

**BLOOD GROUPS
AND
TRANSFUSION**

Third Edition—Third Printing

Blood Groups and Transfusion

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TO
MY WIFE
GERTRUDE

PREFACE TO THE THIRD EDITION

THE LAST three years have witnessed a marked increase of interest in the fields of blood transfusion and blood grouping, owing not only to the outbreak of the war but also to the widespread adoption of "blood banks" in civilian hospitals. Hundreds of new articles on these subjects have appeared since 1939, when the second edition of this book was printed, and during the present year three important monographs have been published in this country—one dealing with blood grouping technic, the other two with the transfusion of blood and blood substitutes. Since there still appeared to be need for a more comprehensive book, containing in compact form the available knowledge concerning both fields, blood grouping and transfusion, this third edition was prepared.

This volume is intended to summarize the present knowledge concerning the theory and technic of blood grouping, to present the applications in clinical, legal and veterinary medicine and in anthropology, and to assemble and integrate the essential facts concerning the transfusion of whole blood, plasma and serum.

The present edition contains two new chapters—one dealing with the transfusion of stored blood and blood substitutes, and the other with the recently discovered Rh factor and its rôle in erythroblastosis fetalis. The remaining chapters have been extensively revised and considerably amplified by the addition of new material, in some cases, chapters have been almost completely rewritten. For example, on account of the increased interest in blood tests evinced by anthropologists, many new data have been added to the sections dealing with the racial distribution of the blood groups and the blood group factors in apes and monkeys, and older observations have been re-interpreted in the light of our more recently acquired knowledge.

An attempt has been made to make the presentation readable to those unacquainted with the subject, but without sacrificing completeness of exposition, as far as possible. Those sections which may be of interest only to specialists are given in small print and may be omitted without loss of continuity. As in the previous editions, not every contribution could be included; an endeavor has been made to review all significant publications, but a number of papers worthy of notice may well have been overlooked.

It is hoped that the section on the transfusion of stored blood and plasma may prove useful in connection with the application in military medicine. The section on transfusion reactions had to be rewritten in view of the newer knowledge concerning intragroup incompatibility.

The author wishes to take this opportunity again to thank all those readers who have made suggestions and comments of which he has made use in preparing this revised edition. He is particularly indebted to several of his colleagues who were kind enough to read and criticize the manuscript and who have thus given him the advantage of their experience. To Mrs. Barbara Mullin and Mr. Isidore Kosofsky he is indebted for their diligent assistance in the preparation of the manuscript material, and to Mr. Charles C. Thomas, as always, the writer wishes to express his appreciation for his cooperation and helpful advice.

A. S. WIENER

Brooklyn, New York
January 2, 1943

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**BLOOD GROUPS
AND
TRANSFUSION**

CHAPTER I

INTRODUCTION

SINCE time immemorial the individuality of all organisms has attracted attention. No two animals, no two plants are exactly alike. The morphological differences of animals have been used as a basis for their classification into groups of similar animals, known as species, but, within each species, morphological differences of a lesser degree exist. Thus, human individuals can be recognized by their features, or even by their voices. And the fact that the finger prints of any individual are different from those of every other human being is accepted as a means of identification in criminal cases.

Some of the differences which determine individuality are inherited, whereas others are caused by the environment. The hereditary variations are determined by genes which are located in the chromosomes. The genes occur in pairs, one member of each pair having been derived from the maternal parent and the other from the paternal parent. There is usually at least one pair of genes controlling each characteristic of the individual. In the *Drosophila melanogaster*, the species whose heredity has been most thoroughly studied, and which has only four pairs of chromosomes, more than 500 distinct pairs of genes have been identified.¹ Since man possesses 24 pairs of chromosomes, the possibilities of variation would seem to be much greater. The genetic make up of an individual determines his "genotype," but since the influence of the germ plasma is modified by the effects of the environment, individuals with the same genotypes do not appear identical. The ultimate composition of the individual resulting from the combined influence of heredity and environment is termed the "phenotype." It is obvious that there is even greater variation among the phenotypes than among the genotypes.

Whereas the morphological species characteristics and individual differences have been known from the earliest times, until comparatively recent years the existence of biochemical differences was not clearly recognized. During the nineteenth century, some blood transfusions were performed on human beings with sheep's blood or the blood of other domestic animals. The occurrence of fatal reactions to such transfusions

¹ By comparing mutations induced by x ray treatment with those occurring naturally. Gowen and Gay [*Genetics* 18: 1 (1933)] have estimated that there are about 2000 distinct genes capable of producing visible effects in *Drosophila*, and in addition there are many other loci occupied by genes vital to the normal morphology and well being of the organism.

caused Landois and others to investigate this problem Landois² found that if human blood is mixed with the blood of other animals *in vitro*, hemolysis or agglutination of the blood cells results This work as well as Bordet's work, in which he immunized animals with the serum or blood of animals of different species, led to the concept of a species specificity of the blood³ From microscopic studies it had also become known that the erythrocytes of animals of different species vary in morphology, i.e., with respect to their size, shape, presence or absence of a nucleus, etc

The first observations on differences between the bloods of normal individuals belonging to the same species were made by Landsteiner⁴ in human beings in 1900 Landsteiner's investigations were prompted by the discovery of serological species specificity, which raised in his mind the question whether similar specific differences presumably of a minor order, existed between individuals of the same species He chose the simplest possible method of investigation namely of mixing the serum of one normal individual with the red blood cells of other normal individuals Instead of the reactions of a minor order that might have been expected, Landsteiner found that in certain cases a marked agglutination of the red blood cells resulted while in other cases the blood cells were entirely unaffected On the basis of the isoagglutination⁵ reaction, Landsteiner was able to divide human beings into three distinct groups⁶ The fourth and rarest group was discovered in 1902 by von Decastello and Sturli,⁷ the latter a pupil of Landsteiner's who continued this work at Landsteiner's suggestion⁸ On account of the constancy of the blood groups, from the outset their constitutional nature was obvious Nevertheless, several subsequent authors attributed the occurrence of the phenomenon of isoagglutination to disease

A continuation of these studies in man and other animals revealed that in several of the higher animals at least, a marked individuality of the blood exists Shortly after Landsteiner's original discovery Ehrlich and Morgenroth⁹ succeeded in demonstrating individual differences

² Landois *Die Transfusion des Blutes* Leipzig (18 5)

³ *Annales de l'Institut Pasteur* 12 683 (1898)

⁴ *Zentralbl f Bakteriöl Parasitenk u Infektionskrankh* 27 35 (1900)

⁵ The term isoagglutination refers to agglutination resulting from mixtures of cells and serum of individuals of the same species heteroagglutination refers to similar reactions between bloods of individuals of different species

⁶ *Wien klin Woch* 14 1132 (1901)

⁷ *Munch med Woch* 49 1090 (1902)

⁸ See Decastello *A Wien klin Woch* 44 (1931) With regard to the circumstances leading to the discovery of the blood groups the reader is referred to Landsteiner's Nobel Lecture *Science* 73 403 (1931)

⁹ *Berlin klin Woch* 37 453 (1900)

in the blood of goats with the aid of immune sera prepared by injecting goats with the blood of other goats. Similarly, in 1910, Todd and White¹⁰ produced immune isoantibodies by means of which the blood of one cow or bull could be identified among the bloods of more than 100 other cattle. The existence of a similar individuality in the blood of chickens was first indicated by the work of Landsteiner and Miller¹¹ in 1924, and in 1930, Todd¹² demonstrated practically a complete individuality of the blood in that species. Evidence that individual differences exist in the blood of other species has been obtained in anthropoid apes, lower monkeys, dogs, cats, horses, sheep, pigs, rabbits and, more recently in mice, rats and pigeons.

In 1911 von Dungern and Hirszfeld¹³ discovered the existence of subdivisions in two of the four human blood groups, and in 1927 Landsteiner and Levine¹⁴ discovered three additional individual properties in human blood, designated by them as M, N and P. Later studies on individual differences in human blood indicate the existence of still further serological differences so that in this respect there is no fundamental discrepancy between the conditions obtaining in human and animal blood. Of the more recently reported individual differences in human blood those determined by the property Rh, described by Landsteiner and Wiener¹⁵ in 1940 have proved to be of clinical significance.¹⁶

The serological differences in the blood cells are purely constitutional in nature, they are determined solely by heredity, and are not influenced by environment. That the four Landsteiner blood groups are inherited was suggested by Ottenberg and Epstein¹⁷ in 1908 and definitely proved in 1910 by von Dungern and Hirszfeld.¹⁸ The exact mechanism of heredity was not determined until 1925, however, when Bernstein¹⁹ solved this problem. Also the more recently discovered serological differences in human blood are inherited according to Mendel's laws, and the same has been found to be true of animal bloods as far as they have been studied. The constitutional nature of the individual differences of the blood has been emphasized by the discovery that the substances which

¹⁰ *Proc Royal Soc B* 82 416 (1910)

¹¹ *Proc Soc Exp Biol and Med* 22 100 (1924)

¹² *Proc Royal Soc B* 106 20 (1930)

¹³ *Zeitschr f Immunitat* 8 526 (1911)

¹⁴ *Proc Soc Exp Biol and Med* 24 600 941 (1927)

¹⁵ Landsteiner and Wiener *Proc Soc Exp Biol and Med* 43 223 (1940)

¹⁶ Wiener and Peters *Ann Int Med* 13 2306 (1940) Levine, Katzin and Burnham *Jour Amer Med Assoc* 116 825 (1941)

¹⁷ *Trans N Y Pathol Soc* 8 187 (1908)

¹⁸ *Zeitschr f Immunitat* 6 284 (1910)

¹⁹ *Zeitschr f indukt Abstammungs u Vererbungs* 37 237 (1925)

characterize the four blood groups are present in almost every tissue of the body, and in most individuals, also in the body fluids and secretions

The manifold applications of all this work in biology, and in clinical and legal medicine prove that it is not merely of academic interest. The application of the Landsteiner blood groups for the selection of blood donors has made blood transfusion a relatively safe procedure and has undoubtedly saved thousands of lives. In forensic medicine this knowledge has been applied for the identification of blood stains and for the determination of non paternity. Data have been collected by studies on the serological properties in various human races and in primates which are of value to anthropology, and contribute to the knowledge of biochemical evolution. Its contribution to serology has been to increase our understanding of the nature of the cellular antigens. The subject should therefore be of interest not only to the immunologist, but also to the physician, the lawyer, the geneticist and the anthropologist.

CHAPTER II

THE FOUR BLOOD GROUPS

When the red blood cells of any normal human individual are suspended in normal saline solution, an even suspension results. If this red blood cell suspension is mixed with the serum of the same individual or of other individuals belonging to the same blood group, the suspension remains apparently unaffected (cf fig 1). On the other hand, if the blood cells of individuals of certain groups are mixed with the serum of individuals belonging to certain other groups, the red blood cells will come



FIG 1 NEGATIVE REACTION
(No agglutination)
Magnified 180



FIG 2 POSITIVE REACTION
(Showing intense agglutination of the
red blood cells)

together in clumps (cf fig 2). This phenomenon of agglutination forms the basis for the division of all human beings into the four groups O, A, B, and AB (cf table 1).

The agglutination of cells when mixed with serum depends upon a specific reaction between two substances, one in the red blood cells (called the *agglutinin*), and the other in the serum (called the *agglutinin*). This reaction is of the same order as the well known agglutination tests in bacteriology, in which case the agglutinin is contained in bacterial cells. The specificity of the reaction may be compared to the specificity of the Widal reaction and other specific agglutination reac

tions commonly used in bacteriology. The technic of blood grouping is simpler than that of the bacteriological tests, and the reactions are so clear-cut that there can be no uncertainty when the tests are properly performed.

According to Landsteiner,¹ the existence of the four blood groups depends upon the presence or absence of two agglutinogens A and B in the red blood corpuscles and two agglutinins, α (or anti-A) and β (or anti B), in the serum (or plasma), such that α is specific for A, and β is specific for B. In table 1 is shown the composition of each of the four Landsteiner blood groups. Several different nomenclatures for the four blood groups have been used, namely, the numberings of Moss and Jansky, and the International Nomenclature, officially recognized by the Health Committee of the League of Nations. The Moss and Jansky numberings are no longer used in scientific publications, and are only given here because they are still being used in a number of in-

TABLE 1

CLASSIFICATION AND COMPOSITION OF THE LANDSTEINER BLOOD GROUPS

International Nomenclature	Jansky Numbering	Moss Numbering	Cells (Agglutinogen)	Serum (Agglutinin)
O	I	IV	—	α and β
A	II	II	A	β
B	III	III	B	α
AB	IV	I	A and B	—

stitutions. The existence of two systems of numbering has resulted in confusion on many occasions, and has probably been responsible for several transfusion accidents. By the use of the International Nomenclature, which corresponds to the agglutinogen content of the red blood cells, such mistakes are prevented.

Levine and Katzin² have recently made a survey of the classifications in use in American hospitals. From their data, there appears to have been a gradual increase in the use of the International Nomenclature. The interest in the medicolegal applications of blood grouping will undoubtedly stimulate the more general use of the International Nomenclature, since in this manner the heredity of the blood properties is presented more directly.³

If the sera of individuals of each of the four blood groups are mixed

¹ *Wien klin Woch* 14: 1132 (1901)

² *Jour Amer Med Assoc* 110: 1243 (1938)

³ In this connection see "Report of the Committee on Medicolegal Blood Grouping Tests [*Jour Amer Med Assoc* 108: 2138, 2215 (1937)] in which the use of the International Nomenclature is advocated

with the red blood cells of individuals of each group, the results will be as shown in table 2. It may be seen that the reactions produced by testing with sera of group A and group B, when considered together, are different for the blood cells of each of the four blood groups. Consequently, the blood group of any individual can be determined by testing his red blood cells with these two sera alone (cf table 3). The serum of group A, which contains the agglutinin β (or anti B), will determine the presence or absence of agglutininogen B. On the other hand, the serum of group B tests for the presence or absence of agglutininogen A. If neither of the two sera react with the unknown blood cells suspension, the individual belongs to group O. If the cell suspension is

TABLE 2

Serum of Group	Agglutinins in Serum	Red Blood Cells of Group			
		O	A	B	AB
O	α and β	-	+	+	+
A	β	-	-	+	+
B	α	-	+	-	+
AB	-	-	-	-	-

+ signifies agglutination

- signifies absence of agglutination

agglutinated by both sera, the individual belongs to group AB. Blood reacting only with serum B belongs to group A, and conversely, blood reacting only with group A serum belongs to group B.

When determining the blood group of an individual, as a rule it is sufficient only to test the blood cells for their agglutininogen content. But in cases of particular importance, or where there is the slightest doubt as to the results of grouping, it is necessary to test also for the agglutinin content of the serum. According to Landsteiner's rule those agglutinins and only those are present in the serum for which there is no correspond

TABLE 3

Unknown Cell Suspension of Group	A serum (anti B)	B serum (anti A)
O	-	-
A	-	+
B	+	-
AB	+	+

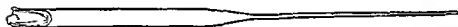
ing agglutinin in the red blood cells, e.g., the serum of a group A individual contains only agglutinin β , not agglutinin α . The examination of the serum for its agglutinin content, therefore constitutes a valuable check on the technic. The tests are performed by mixing the unknown serum with known cell suspension of groups A and B. If both cell suspensions are agglutinated, the unknown serum contains both agglutinins, and consequently the individual belongs to group O. In a similar way the other groups are determined (cf. tables 1 and 2).

TECHNIC OF BLOOD GROUPING

A Stock Testing Sera As has just been pointed out, only two testing



FIG. 3



A

Sterile Pipette and Nipple One end has been loosely packed with cotton; the tip has been sealed and the pipette then sterilized in a hot air oven. Before use the tip of the pipette is broken off and the outside of the capillary is flamed.



B

Sterile 2 c.c. vial



C

Sterile vial containing serum and hermetically sealed

sera are necessary, one from an individual of group A and the other from an individual of group B. The sera selected should be of high titer so that they react with bloods of low sensitivity (cf. page 18), such as occur in groups A and AB (subgroups A_2 and A.B), particularly in infants. With a weak serum only the more sensitive blood cells will react, and errors in grouping have resulted in this way.

It has been found that the serum of individuals suffering from certain pathological conditions causes a high degree of pseudagglutina

tion and autoagglutination which may be mistaken for true isoagglutination (cf pages 36 and 39) Furthermore, the titer of the agglutinins in very young children or old individuals is usually lower than that of young adults and middle aged individuals (cf fig 9) For these reasons, preferably healthy young adults or middle-aged individuals should be selected for obtaining testing sera

The blood is best distributed in test tubes and allowed to coagulate The clotted blood is rimmed with a stirring rod, and the serum can be separated either by centrifuging the blood, or by allowing the blood to stand in the ice box over night The serum is then transferred to ampoules, with the aid of capillary pipettes (cf figure 3) All operations must, of course, be done under sterile precautions In the writer's laboratory the sealed ampoules are placed in a water bath at 56°C for 20 to 30 minutes and then labelled with the group and date It is convenient to use ampoules of only 1 c c capacity because if the serum is kept in large ampoules, it may be contaminated by repeated withdrawals High titered serum which has thus been properly stored in the ice box, retains its potency for a long time Various chemical preservatives have been suggested but they are unnecessary, and it is preferable to store unmodified serum One of the preservatives suggested is phenol to 9 parts of serum is added 1 part of 5 per cent phenol in normal saline solution Rosenthal⁴ has suggested the following preservatives, which at the same time, color the two sera differently, thus aiding in their identification To each c c of group A serum is added 0.01 c c each of 1 per cent aqueous solution of neutral acriflavine and 0.5 per cent aqueous solution of basic fuchsin, and to each c c of group B serum is added 0.01 c c of 1 per cent aqueous solution of brilliant green Another useful preservative is merthiolate, one part of the 1 per cent solution being added to 50 parts of serum

Some workers have suggested the storage of grouping sera in the dried form, for example, sera dried in the Flosdorf Mudd apparatus However, the use of special apparatus and additional work is required for drying sera, and, besides, the dried sera have no superior keeping qualities as compared to the liquid sera

For obtaining stock typing sera it is preferable as Thomsen suggests to allow the blood to stand in the ice box overnight and to draw off the serum at low temperature (if necessary by centrifugation) so that if any auto-agglutinins are present (cf page 39) they will be bound by the blood corpuscles as far as possible and thus removed from the serum If the serum has any rouleaux forming properties it should be diluted with an equal volume of saline before use

⁴ *Jour Lab and Clin Med* 16 1123 (1931)

⁵ Ives and van Roogen *Brit Med Jour* 2 841 (1939) also cf Flosdorf and Mudd *Jour Immunol* 34 469 (1938)

Sera may also be purchased from certain commercial houses. Since occasionally preparations are encountered which are not reliable,⁶ each vial of serum should be tested for its potency and correct labelling before use.

B The Blood Cell Suspension The blood cell suspensions used should be fresh (cf page 20). A few drops of blood are obtained from the finger or ear lobe and suspended in several c c of normal saline solution⁷ (to which may be added also a small amount of 3 per cent sodium citrate solution). This may be used directly, or if desired, may be washed once as follows. The suspension is centrifuged and the supernatant solution discarded. The red cells are then resuspended in normal saline to make a 1 to 2 per cent suspension (in terms of blood sediment), as judged by the eye.

If one desires to examine both cells and serum, 1 to 2 c c of blood are collected in a test tube by means of a venepuncture, or a smaller quantity from the finger or ear lobe in a capillary tube. The blood is allowed to clot, it is then rimmed and centrifuged. The serum is pipetted off into a separate tube to be tested later on, and the blood clot is shaken up with normal saline solution. Some of the blood suspension thus formed is pipetted off into another tube, the blood cells are then washed once and resuspended in normal saline solution to make a 1 to 2 per cent suspension. When the time element is important, as before transfusions, the blood can be collected into citrate solution (cf page 61) or mixed with a small amount of powdered potassium oxalate and the plasma and blood cells separated by centrifuging can be used for the tests. The presence of the small amounts of citrate or oxalate required does not interfere with the reactions. Or else, the unmodified blood can be defibrinated by whipping it with wooden applicators.

In order to determine the agglutinin content of unknown sera it is necessary to prepare cell suspensions of known groups. If individuals of group A and group B are not always available, the blood may be kept for several weeks in the ice box by preserving it in the solution recommended by Rous and Turner.⁸ 3 parts of sterile, whole blood are mixed with 2 parts of 3.8 per cent sterile, sodium citrate solution, and 5 parts of 5.4 per cent sterile, glucose solution. When needed, a small amount of this blood may be removed with a sterile pipette and suspended in normal saline solution, this suspension being washed once as described.

⁶ *Coca Jour Lab and Clin Med* 16 405 (1931)

⁷ The physiological⁹ (or normal) saline solution used for blood cell suspensions is prepared by dissolving 8.5 to 9.0 grams of sodium chloride in a liter of distilled water. It may be found more convenient to prepare a stock 10 per cent saline solution from which the dilute solution can be prepared as needed.

⁸ *Jour Exper Med* 23 219 (1916)

above for the fresh cell suspension. However, blood cells keep as well or better when preserved as a clot in its own serum.

On the other hand, saline suspensions must be quite fresh when used since, apart from bacterial contamination even at low temperatures changes occur in the properties of the erythrocytes that may give rise to false agglutination (cf. page 46) and weaken the specific reactions.

TABLE 4

EXAMINATION OF UNKNOWN BLOOD CELL SUSPENSIONS WITH KNOWN TEST SERA

Bloods of	Reactions with A Serum	Reaction with B Serum	Group
Unknown 1	—	—	O
Unknown 2	—	—	O
Unknown 3	—	+++	A
Unknown 4	—	++	A
Unknown 5	+++	++	AB
Unknown 6	—	—	O
Unknown 7	—	+++	A
Unknown 8	+++	—	B
Unknown 9	—	—	O
Unknown 10	—	—	O
CONTROLS			
Group O	—	—	
Group A	—	+++	
Group B	+++	—	

The strength of the reactions is indicated by crosses for instance

Positive +++ one large clump complete agglutination + clumps just visible macroscopically

Negative — microscopically homogeneous suspension

According to Sandstrom* blood suspensions can be preserved by the addition of formalin in a concentration of 1:1000.

C. The Grouping Tests The blood grouping tests may be performed in several ways. According to the technique recommended by Landsteiner,⁹ one drop each of unknown cell suspension, saline and testing serum are mixed in a small test tube (inside diameter 7 mm) which is then allowed to stand. The reactions are usually visible within a few minutes but the final reading should be made after an hour. A small drop of the mixture is transferred to a glass slide by means of a stirring rod,

* *Zentralb. Bakter.* 113: 1 (1929)

⁹ Cf. page 905 in *The Newer Knowledge of Bacteriology and Immunology*, Edited by Jordan and Falk, University of Chicago Press (1932)

and examined microscopically under low power. As a control, blood suspensions of known groups should be included in each experiment. By way of illustration, a sample protocol is given in table 4.

The reactions can be expedited and intensified by centrifuging the tubes at about 2000 revolutions per minute, for about one minute.¹¹ The tubes are then replaced in the rack, which is shaken until the negative control blood cells are evenly suspended. The reactions are read with the naked eye (Schiff, cf. fig. 4).

A third method of performing the tests, which is convenient when only one or two bloods are to be examined, is carried out by mixing the

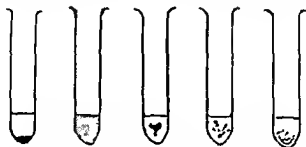


FIG. 4 BLOOD GROUPING BY THE CENTRIFUGE METHOD

Test tube 1 The mixture of cell suspension and serum is centrifuged and as a result the blood cells are packed at the bottom of the tube.

Test tube 2 After shaking the sedimented cells break up into an even suspension—Negative reaction.

Test tube 3 Shaking fails to separate the packed cells which float about in one clump—Strong positive reaction.

Test tube 4 Positive reaction of moderate strength.

Test tube 5 Same as 4 after standing a short time.

cell suspensions and serum directly on a glass slide (cf. fig. 5). One drop of group A serum is placed on the left end of the slide and 1 drop of group B serum on the right end of the slide, and 1 drop of unknown cell suspension is mixed with each of these sera. The slide is then tilted back and forth for 3 to 5 minutes, after which time the drops may be covered with cover slips, to facilitate examination under the microscope. In applying this method, one must take into account the possibility of pseudoagglutination (cf. page 36), since the serum is only half diluted.

By using glass slides or porcelain plates with a number of wells it is possible to test many bloods simultaneously by an open slide method. The paraffin ring slides used in the Kline test for syphilis are well suited for this purpose.¹² They are pre-

¹¹ Schiff, *Die Technik der Blutgruppenuntersuchung*, Page 23, Julius Springer, Berlin (1932); Levine and Mabee, *Jour. Immunol.* 8: 428 (1923).

¹² Kline, *Microscopic Slide Precipitation Tests for Syphilis*, 99 pp., William & Wilkins Co., Baltimore (1932).

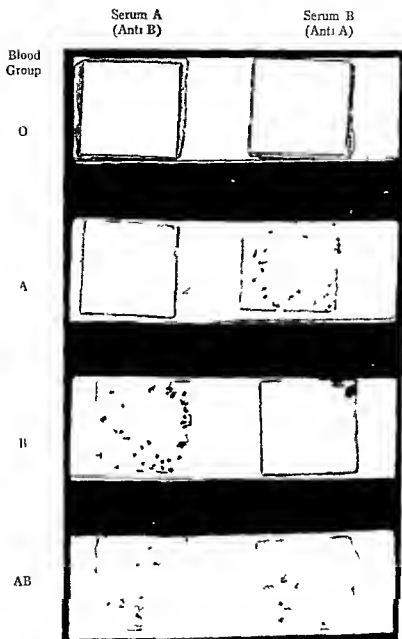


FIG. 5 BLOOD GROUPING ON GLASS SLIDES
(Actual size)

pared as follows. Glass slides (2 x 3 inches) are thoroughly cleaned and rubbed on both sides with paste prepared from scouring powder (e.g. Bon Ami powder). When the paste is dry it is completely removed from the slide with a clean cloth. The instrument used for making the rings (Green) consists of a soft iron wire (No. 28) which has been wound twice around a test tube to form a double loop

about 15 mm in outside diameter, the ends of the wire being twisted to form a doubled stem which is inserted into a wooden handle or a hemostat. The wire is covered by closely winding linen thread (no. 12) about it. To make the paraffin rings the instrument is dipped into melted paraffin (about 120°C), drained quickly by touching the side of the dish and then the ring is made by placing the loop on the cleaned glass slide. The capacity of each well being about 0.5 c.c., in making the grouping tests small drops of serum and cell suspensions are used (approximately 0.25 c.c.). The serum and cell suspension are mixed by rotating the slide rapidly on

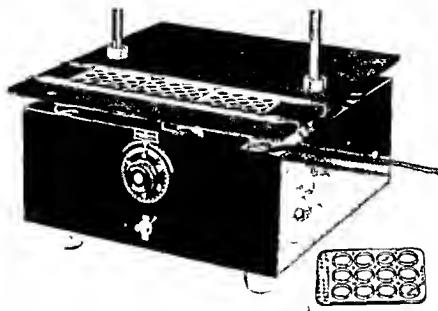


FIG. 6 BOERNER SHAKING APPARATUS SHOWING WELL SLIDES
IN POSITION FOR SHAKING
(Courtesy of A. H. Thomas Co.)

a flat surface or by tilting the slide back and forth for 4 to 5 minutes after which time the reactions are read both macro- and microscopically. By using a special tray which can hold 3 of the paraffin ring slides as many as 36 tests can be readily done simultaneously but no more than this number should be attempted at one time lest evaporation interfere with the reactions. In using this technique of course one must be aware of the possibility of pseudoagglutination as in the common slide method. Using serum diluted 1:2 or 1:3 will circumvent this drawback.

The performance of multiple tests by the open slide method has been simplified by the appearance on the market of prepared slides each with 12 wells. When such slides are used it is only necessary to rinse them off with water and to dry them in the air or by wiping before carrying out additional tests. The tests can be further facilitated by using a shaking machine (cf. figure 6).

The method recommended by Landsteiner is particularly advisable when a number of bloods is to be examined at the same time. Moreover,

tests made in tubes can be examined repeatedly over long periods of time, and the dilution with normal saline solution as a rule prevents the occurrence of pseudoagglutination. The centrifuge method has the advantage of speed, and by this method also many specimens of blood may be examined simultaneously. The common slide method is especially convenient when only 1 or 2 specimens are to be examined, as when testing blood before a blood transfusion. A number of workers perform the grouping tests by the hanging drop technic which is commonly employed for bacterial agglutination tests. This method is too tedious for routine tests but may prove advantageous when the amount of material available for examination is small as with blood stains (cf page 414). The tests by any method are usually performed at room temperature. Very low temperatures are to be avoided because of the danger of non specific reactions (cf page 39).

D Titration of Sera For the selection of testing sera (cf page 10), and for other special purposes, it is necessary to determine the strength of the sera. One method of doing this is by titration. The principle in titration is to mix progressively higher dilutions of the serum with a constant amount of sensitive red blood cells. The titer of a serum is given by the reciprocal of the highest dilution of the serum at which agglutination still occurs. Thus, if the highest dilution of the serum at which agglutination occurs is $1/64$, the titer of the serum is said to be 64.

The titration of the serum may be set up in small tubes as follows. In the first tube is placed 0.2 c.c. of undiluted serum and in each succeeding tube is placed 0.2 c.c. of normal saline solution. To the second tube is now added 0.2 c.c. of serum and the serum and saline in this tube are mixed. 0.2 c.c. of the mixture is transferred to the following tube and the procedure is then repeated for each of the subsequent tubes discarding the 0.2 c.c. of diluted serum obtained from the last tube. Each tube now contains 0.2 c.c. of progressively doubled dilutions of serum. To each tube is added 0.2 c.c. of a 2.5 per cent suspension of sensitive red blood corpuscles. The mixtures of diluted serum and blood cell suspension are allowed to stand with occasional shaking for 2 hours at room temperature after which time the contents of each tube is examined for the presence or absence of agglutination. A sample protocol of a serum titration would read as follows:

<i>Serum Dilution</i>	1 1	1 2	1 4	1 8	1 16	1 32	1 64
<i>Reaction</i>	+++	+++	++±	+±±	+±	±	—

The titer of the serum in this case is 32.

The measuring pipettes used for making the dilutions of serum should be equipped with a small loose plug of non absorbent cotton (Thomsen). This is to prevent saliva from getting into the pipette since inhibiting substances may be present in the saliva (cf page 274). The dilutions can also be prepared with capillary pipettes and nipples measuring the quantities by drops. Although the principle of titration is simple in practice the results are not entirely satisfactory for

even with careful work a difference of 1 and sometimes even 2 tubes cannot be avoided¹³

For the selection of testing sera an abbreviated titration using only dilutions 1 1 10 1 20 1 40 and 1 80 is sufficient. A satisfactory serum will give reactions visible to the naked eye in dilution 1 20. However, as it is best to dilute the testing sera with an equal volume of saline before use (cf page 11) the minimum acceptable titer should be 40. When testing the potency of group B sera cells of the less sensitive subgroup A_2 (cf table 198) should be used; if the subgroup of the test cells is not known the titer of the undiluted serum should be no less than 80 units. In addition to having a high titer the typing sera should give rapid and marked clumping under the conditions of the tests. As a rule there is a marked correlation between the titer of the serum and the strength of the reaction, but sera of high titer are occasionally encountered which give only moderate clumping.

In the method of titration described above the other constituents of the serum as well as the isoantibodies are diluted by the saline. If instead of saline one takes as a diluent the individual's own serum or serum of the same group absorbed so as to remove the isoagglutinins the titer values are considerably lower as a rule.^{12a} Failure to take this fact into account led Levinson and Cronheim¹⁴ erroneously to ascribe the reduction in isoagglutinin titer they observed when sera or plasma of a different group was used as a diluent in titrations solely to the presence of group substances in solution (cf page 150).

Subgroups of Group A and Group AB. As has just been mentioned there are two sorts of A agglutinogens A_1 and A_2 , which differ considerably in sensitivity (cf Chapter XI), whereas, in adults there is little or no variation in the sensitivity of the agglutinin B in different individuals. Furthermore, the reactivity of the agglutinin A is often weaker in group AB. The relative average sensitivity of the A agglutinogens in the four subgroups can be represented as follows: $A_1 > A_1B > A_2 > A_2B$. As already mentioned the A receptor in adults of subgroup 1 B, and in infants of subgroup A_2 is well may be so weak that it can only be detected with the most potent anti A sera. With regard to blood of so-called subgroup A_1 , see page 216.

FACTORS AFFECTING THE ISOAGGLUTINATION REACTION

A. Temperature. It has been found that the highest titer at which agglutination will occur when a given serum is mixed with the same sensitive blood cells varies with the temperature at which the tests are performed. In figure 7 are presented the titers of the A agglutinin in seven different sera of group O at temperatures ranging from 0°C to 60°C. It can be seen that the titer of each serum is lower at the higher temperatures, and that sera of very low potency fail to act at the higher

¹³ See the report of the *Conference de Laboratoire sur les Groupes Sanguines* Health Committee of the League of Nations CII 835 Geneva Sept 15 1930.

^{12a} Lubinski *Jour Immunol* 44 95 (1942). Cf Wiener and Derby *Arch Derm and Syph* 39 999 (1939).

¹⁴ *Jour Amer Med Assoc* 114 2097 (1940).

temperatures even if undiluted. The average normal adult serum will give distinct reactions at 37°C . Distinct agglutination rarely occurs above 55°C even with sera of the highest titers.

With respect to its sensitivity to variations in temperature, the isoagglutination reaction resembles hemagglutination by other natural antibodies. The stronger effect at low temperatures indicates the exothermic nature of the reaction (Landsteiner). Exceptional are the reactions produced by certain sera containing irregular isoagglutinins (cf. page 46), which act best at higher temperatures.

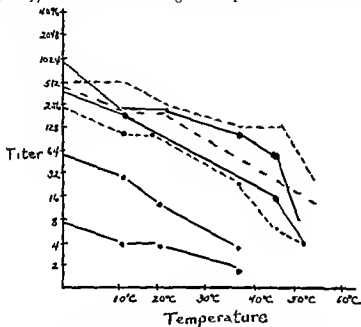


FIG. 7. VARIATION OF TITER OF SERUM WITH TEMPERATURE
(Showing the titer of the α agglutinin in seven
different O sera)
(After Kettel)

B Velocity of Reaction According to the classic conception, agglutination and precipitation reactions proceed in two separate stages: (1) the specific combination between the antigen and antibody, and (2) a non-specific stage of aggregation of the sensitized particles in which electrolytes play a rôle. Thus the binding of agglutinins by red cells is said to cause an increase in the cohesive force between them. The reduction of the negative surface charge of the suspended cells below a certain critical potential by salt in the medium results in the agglutination of sensitized but not of unsensitized cells. Marrack¹⁵ has sug-

¹⁵ Marrack *Spec. Rep. Ser. No. 194* Med. Res. Council London 1934.

gested, however, that the molecules or particles of antigen and antibody molecules alternately combine to build up a three-dimensional lattice by virtue of the presence in the antigenic particles and antibody molecules of several combining groups (cf. Heidelberger and Kendall,¹⁴ also see papers quoted in Wiener and Herman¹⁷)

The "first stage" of the reaction can be assumed to occur rather rapidly, the speed of agglutination depending mainly on the rate at which the sensitized cells are brought together. For example, in tests made in tubes the reactions do not reach their *maximum* intensity until sedimentation is complete (usually after 1 to 2 hours), but the agglutination time can be shortened to 1 to 2 minutes by centrifuging the tubes in order to pack the cells together more quickly.

C Concentration of the Cells This is another factor which influences the agglutination reactions. If an excess of cells is used they may absorb all the agglutinins present in weak or diluted sera but fail to agglutinate with such sera.

D Proportion between Cells and Serum, Dilution If parallel titrations are set up with the same serum and test cells, in one titration mixing one drop of each serum dilution with a drop of two per cent blood suspension, in the other 10 drops (or 0.5 c.c.) of each serum dilution and a drop of a one per cent suspension, agglutination will occur in higher dilutions of serum by the latter technic than by the former. From the proportion between cells and serum one might expect that the titer by the second technic will be 20 times as high. Actually Candela¹⁸ has shown that the ratio between the titers in such experiments is only about 8 to 1. The discrepancy is due to the difference in volumes in which the two titrations are carried out, the clumping being weakened when the cell suspensions are very dilute. At any rate Candela's technic of using a large ratio of serum to cells has proved particularly advantageous for grouping tests on old dried bones, by increasing the range of serum dilutions (cf. page 320).

From the above, it is evident that when the titer of a serum is given it is important to describe the exact procedure followed. When titrating test sera, obviously the technic used should be the same as or comparable to that which will be followed in the actual tests.

E Age of the Blood Suspension If the red blood cell suspension is kept in the ice box, its sensitivity to agglutination will gradually decrease, and the higher the temperature the more rapidly will this occur.

¹⁴ Heidelberger and Kendall *Jour Exp Med* 61: 559 (1935); 62: 46, 699 (1935).

¹⁷ Wiener and Herman *Jour Immunol* 36: 255 (1939).

¹⁸ Candela *Amer Jour Phys Anthropol* 27: 365 (1940). Cf. Wiener, Zinsser and Selkowitz *Jour Immunol* 28: 431 (1934); *Lancet Arch Path* 31: 455 (1941).

Schiff and Hubener¹⁹ found a marked variation in the sensitivity of bloods that had been kept only one day, due to varying losses in sensitivity. It is therefore manifestly advisable to perform all blood grouping tests on fresh cell suspensions.

As already mentioned, blood cells retain their sensitivity far better when kept in concentrated than in dilute suspensions. Blood to be stored or shipped through the mails is best taken from the vein and placed in sterile dry tubes, as for a Wassermann test. The sensitivity of old and partly hemolyzed blood may be restored, in part, by washing with saline solution and then resuspending.

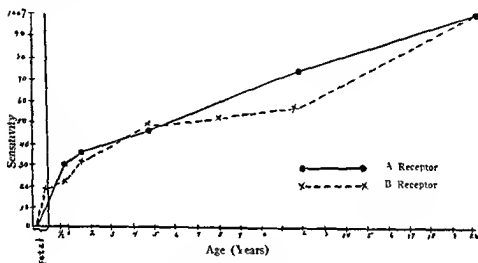


FIG. 8. DEVELOPMENT OF THE AGGLUTINOGENS (After Kemp)

DEVELOPMENT OF THE BLOOD GROUPS

Many studies have been made to determine the manner of establishment of the blood groups.

By studying the sensitivity of the red blood cells to agglutination at different ages, Kemp²⁰ was able to show that the agglutinogen could first be demonstrated in the red blood cells of a fetus, 37 days of age, and that the sensitivity of the cells increases throughout antenatal and postnatal life up to the age of 20 years after which time it remains constant (cf. fig. 8). He found that the red blood cells of the new born infant generally have only 20 per cent of the sensitivity to agglutination that adult blood cells have. Thus, a serum with a titer of 500 against adult corpuscles, would usually agglutinate the corpuscles of the new born infant only up to a dilution of 1/100.

¹⁹ *Zeitschr. f. Immunitats* 45: 207 (1926)

²⁰ *Acta pathol. et microbiol. scand.* 7: 146 (1930)

Thomsen and Kettel²¹ determined the titer of the agglutinins in the sera of individuals of various ages. The results of their investigations are shown in figure 9. The agglutinins are usually not developed at birth, but rapidly increase in titer thereafter up to the age of puberty, after which time the titer gradually diminishes. The curve given in the figure represents the variation of the *average* agglutinin titer of the serum with age. Among individuals of the same age, there is also

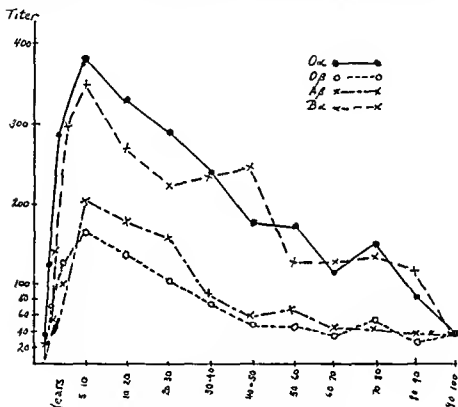


FIG. 9 DEVELOPMENT OF THE ISOAGGLUTININS
(After Thomsen and Kettel)

considerable variation in titer of the agglutinins as was first pointed out by Schiff and Mendlowicz.²² The observations of these authors were confirmed by Kettel,²³ who determined the agglutinin titers of 575 different sera of normal adult individuals against known cell suspensions of groups A and B. Figure 10 graphically represents the per

²¹ *Zeitschr. f. Immunitats* 63: 67 (1929)

²² *Zeitschr. f. Immunitats* 43: 1 (1926)

²³ Kettel K. *Undersøgelser over Ruldehaemagglutinin i Menneskeserum*. Levin and Munksgaard, Copenhagen (1930)

centages of adult individuals with varying titers of the agglutinins. The curves are similar in form to those obtained by Schiff and Mendlowicz. It may be seen that the average titer of the α agglutinin is higher than that of the β agglutinin. This difference in titer of these two agglutinins is present at all ages (cf. fig. 9).

While, as a rule, agglutinogens are demonstrable at birth, only about

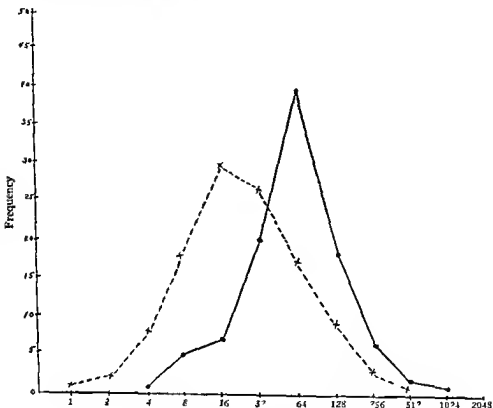


FIG. 10. VARIATION OF ISOAGGLUTININ TITER AMONG NORMAL ADULTS
 (—) α agglutinin
 (---) β agglutinin
 (Traced from data of Kettel)

half of all new born infants have demonstrable agglutinins. According to Hirsfeld,²⁴ whatever agglutinins the child possesses at birth were derived from the mother by filtration through the placenta. Therefore, no new born child possesses agglutinins or hemolysins that act on the moth

²⁴ See Hirsfeld L. *Konstitutionserologie und Blutgruppenforschung* Page 144 Julius Springer Berlin (1928). Also see Hirsfeld and Zborowsky *Klin. Woch.* 4: 1152 (1925).

TABLE 5
COMPARISON OF AGGLUTININ CONTENT OF MATERNAL AND UMBILICAL CORD BLOOD
(After Polayes, Lederer and Wiener)

Group of Mother	Number of Cases Examined	Number of Children								Incompatibility		
		O, o	O, α	O, β	O, αβ	A, o	A, β	B, o	B, α	AB, o	I Number of cases of Cord Serum Agglutinating Cells of Mother	II Number of cases of Mother's Serum Agglutinating Cells of Child
O, αβ	187	25	11	5	72	22	25	11	16	0	0	74
A, β	200	26	0	25	0	70	50	19	0	10	0	29
B, α	84	11	7	0	0	16	0	12	31	7	0	23
AB, o	23	0	0	0	0	15	0	8	0	6	0	0
Totals	500	62	18	30	72	123	75	50	47	23	0	126

er's red blood cells.²⁵ In a study of the agglutinin and agglutinin content of 500 maternal and 500 umbilical cord bloods, not a single exception to this rule was found (cf. table 5).²⁶ The studies of Thomsen²⁷ have yielded similar results.

Smith²⁸ studied the agglutinin and agglutinin content of the blood of infants from day to day during the first few weeks of postnatal life. He found that in most cases, whatever agglutinins were present at birth diminished in titer or disappeared during the first ten days of life, after which time new agglutinins appeared. This was undoubtedly due to the loss of the maternal agglutinins, followed by the production by the infant of its own.

The placental transmission of isoagglutinins from mother to foetus is of interest, since it clearly illustrates the behavior of antibodies in general. For example, syphilitic reagins present in the serum of the newborn child have been shown to be derived from the mother by filtration through the placenta. In the course of a week to a month, these reagins gradually disappear, and then, if the infant actually has syphilis, it begins to elaborate its own reagins. Further evidence that isoagglutinins and other antibodies present in the blood of newborn infants are derived from the mother by placental filtration has recently been provided by Wiener and Silverman,²⁹ who made parallel titrations of isoagglutinins and other hemagglutinins and syphilitic reagins in maternal and umbilical cord blood, and found the ratios between the titers to be relatively uniform. In this way also an index of permeability or coefficient of distribution for antibodies for the human placenta was established at approximately 8:1 to 12:1. The question has also been investigated by Sherman, Hampton and Cooke³⁰ who, however, found a somewhat wider variation in the ratio.

A question of considerable importance, particularly in medicolegal work, is whether agglutinogens absent from the blood cells at birth can appear later on. In a study on a short series of cases in 1928, Smith³¹ claimed to have observed the appearance of additional agglutinogens after birth in two cases, but subsequent workers have failed to confirm his observations despite studies on much larger series (cf. Hyman-Parker³²). Smith's results are probably due to failure to take into account the subgroups. While A₁ blood of infants gives reactions intermediate in intensity between those of A₁ and A₂ adult blood, the blood

²⁵ Cherry and Langrock, *Jour Amer Med Assoc*, 66: 626 (1916), de Biasi, *Jour Amer Med Assoc* 81: 1776 (1923).

²⁶ Polayes, Lederer and Wiener, *Jour Immunol* 17: 545 (1929).

²⁷ *Zeitschr f Rassenphysiol* 5: 122 (1932).

²⁸ *Amer Jour Dis Child* 36: 54 (1928).

²⁹ *Jour Exp Med* 71: 21 (1940).

³⁰ *Jour. Exp Med* 72: 611 (1940).

³¹ *Amer Jour Dis Child* 36: 54 (1928).

³² *Jour Immunol* 43: 1 (1942).

of new born infants of subgroup A gives weak reactions and is comparable to adult A₂ blood (cf page 216) so that the reaction could easily be missed, unless potent sera are used (Forer, unpublished)

DEFECTIVE BLOOD GROUPS

By defective blood groups is meant the occurrence of bloods lacking their full complement of isoagglutinogens and isoagglutinins, such as would be expected according to the Landsteiner rule. Thus, cases of individuals with group A cells but no agglutinin in the serum (A₀) or individuals of group O with only α agglutinin (O, α), have been described. Although the occurrence of this anomaly is very frequent in new born infants (cf page 24), it rarely occurs in adults and most of the cases which have been reported were probably due to weak reactions which were overlooked. Thus, Grove,³³ in a study of more than 2000 bloods failed to find a single deficient reaction. Thomsen³⁴ however, found five defective blood groups among 3500 individuals. In a study of the blood of close relatives of these individuals it was shown at least in two cases, that considering the laws of heredity, it was the agglutinin and not the agglutinogen which was lacking: for instance one blood A₀ proved to belong to group A and to lack agglutinin β not to group AB, and deficient in agglutininogen B.

THE ABSORPTION OF ISOAGGLUTININS

Landsteiner's theory that the four blood groups are due to two isoagglutinogens A and B, in the red blood cells and two isoagglutinins α and β , in the serum can be readily confirmed by several simple absorption experiments. Thus group B serum contains an agglutinin absorbable by group A but not by group B cells and similarly the agglutinin in group A serum is absorbed by group B but not by group A cells. Now, when the tests are performed with group O serum it will be found that the A cells will remove the α agglutinin. B cells remove the β agglutinin and group AB cells will remove both agglutinins. The lack of ability of group O cells to absorb either agglutinin corresponds to their inagglutinability by serum of any group.

As a matter of fact, it was found not infrequently that the absorption of O serum with either A or B cells would cause a distinct diminution in titer of the heterologous agglutinin. This could be due to a non specific absorption,³⁵ or one might assume that the two agglutinins are partly bound together.³⁶

³³ Cited after Landsteiner in *The Newer Knowledge of Immunology and Bacteriology* page 899

³⁴ *Zeitschr f Immunitäts* 57 301 (1928)

³⁵ Thomsen *Zeitschr f Immunitäts* 70 1 (1931)

³⁶ Landsteiner and Witt *Jour Immunol* 11 221 (1936)

A suitable technic of performing the absorptions is as follows. The blood cells necessary for the absorption experiment are obtained by mixing blood with 5 per cent citrate solution, in the proportion of about 5 to 1, to prevent clotting. The plasma is separated by centrifuging the citrated blood, and the sedimented cells are washed 2 or 3 times with normal saline. The washed blood cells are then packed by centrifuging. The serum is obtained in the usual manner, but is inactivated by keeping it at 56°C for half an hour, to prevent hemolysis. One part of packed washed blood cells is mixed with 2 or 3 parts of serum, and the mixture is allowed to stand 1 hour at room temperature after which time the serum is separated by centrifuging. The absorbed serum is then tested against known cell suspensions to determine whether or not the agglutinin in question has been removed. If any of the absorbable agglutinin remains, more of the packed cells should be added and the process repeated until the absorption is complete. Partial absorption of the agglutinins is demonstrated by comparing the titer of the serum before and after treatment with the absorbing blood.

Preparation of Agglutinin Solutions Landsteiner³⁷ showed that it is possible to reverse the process of absorption of the agglutinin, and thus obtain purified solutions of the isoagglutinins. The method is based on the fact that the absorbed agglutinins are set free from the agglutinated cells at higher temperatures.

The technic as described by Landsteiner and Miller³⁸ is as follows: "5 c.c. of strongly agglutinating human serum, group A or B, were mixed with 5 drops of packed, washed human blood cells of group B or A, respectively, and allowed to stand about 2 hours at room temperature, during which time they were occasionally agitated. The cells were then separated by centrifugalization and washed 3 times with salt solution. The sediment, which showed strong agglutination, was taken up in about 0.6 c.c. of salt solution and placed in a water bath at 56°C, for not longer than 5 minutes, during which time it was shaken. The clumps were usually broken up by this treatment. The tube was then placed in a large centrifuge cup filled with water at 56°C and centrifuged a short time at high speed to throw down most of the sediment as quickly as possible. The supernatant fluid was then removed and centrifuged a longer time unheated. The supernatant fluid now obtained is the agglutinin solution ready for use."

Such agglutinin solutions which contain the isoagglutinins in purer form are used for special purposes (cf. page 332).

ISOHEMOLYSIS

When grouping tests are carried out with fresh human serum, not infrequently hemolysis as well as agglutination of the test cells is observed, as evidenced by the increase in transparency of the mixture. This phenomenon, which does not occur with stored or inactivated serum, is dependent on the presence of complement in the fresh serum. As a rule, when hemolysis occurs, the agglutination of the cells is less marked and at times may not be discernible. However, hemolysins in-

³⁷ *Münch med Woch*, No 46, 1905 (1902)

³⁸ *Jour Exper Med* 42: 853 (1925)

variably correspond in specificity to the isoagglutinins, and in grouping and compatibility tests, the two reactions should be considered equivalent. Sera of individuals of group AB naturally contain no isohemolysins, sera of group A can only contain hemolysins for group B and AB, while sera of group B can contain hemolysins only against bloods of groups A and AB, and sera of group O hemolysins for bloods A, B and AB. In the hemolysis reaction, blood of group A, for example, combines specifically with the α isoantibodies in group B or group O serum and becomes "sensitized" to the complement, the latter being non-specific in its action.

Like the normal isoagglutinins, the isohemolysins were first considered to be pathological. It has been shown, however, that these isohemolysins are normal physiological structures,³⁸ whose function or biological significance, just as in the case of the isoagglutinins, has not yet been elucidated.

Most investigators have found that isohemolysins occur only in about 30 per cent of the cases where it might be expected according to the Landsteiner rule of the blood groups, even though fresh serum is used.³⁹ It has been shown by Hesser⁴⁰ that if the test cell suspensions have been kept for three to four days in the ice-box, they are more readily hemolyzed, and that hemolysis now occurs in almost every case where it would be expected. Hemolysis is also favored by the use of an excess of serum. Group O cells, naturally, are not hemolyzable. If the serum is warmed to 56°C for half an hour, or allowed to stand several days at room temperature, the complement will be destroyed and hemolysis can no longer occur. For this reason only fresh serum can be used if it is desired to demonstrate isohemolysins.

Not all investigators have succeeded in reactivating human sera after destruction of the complement.⁴¹ In the experiments of Thomsen and Thisted,⁴² however, reactivation was regularly successful when fresh human serum of group AB, or guinea-pig serum was added to supply fresh complement.⁴³

Isohemolysin tests are performed by adding a small quantity of cell suspension to undiluted fresh serum in a test tube and incubating the mixture at 37°C. The mixtures are centrifuged or allowed to settle so that the unhemolyzed cells may be

³⁸ Landsteiner and Leiner, *Zentralbl f Bakteriöl, Parasitenk, u Infektionskrankh* 38: 548 (1905); Moss, *Bull Johns Hopkins Hosp* 21: 63 (1910).

³⁹ Moss loc cit, Grafe and Graham, *Münch med Woch* 58: 2257 and 2338 (1911), Jervell, *Jour. Immunol* 6: 445 (1920); etc.

⁴⁰ Hesser, *Acta med scandinav* 57: 415 (1922), 61: Suppl (1925).

⁴¹ Cf. Moss, Grafe and Graham.

⁴² Kolle, Kraus, and Uhlenhuth. *Handbuch der pathogenen Mikroorganismen* Page 1231 3rd Ed II.

⁴³ In these experiments, the sera were inactivated at 50°C for 30 minutes, or at 55°C for 8 to 10 minutes.

separated off. The degree of hemolysis can be determined from the depth of the red color of the supernatant fluid.

Whereas sera containing potent anti B hemolysins will hemolyze all B blood to about the same degree, bloods containing A agglutinin vary in their susceptibility to anti A hemolysins, depending upon the subgroup. Thus, Landsteiner and Levine and Thomsen have shown that when fresh group O and group B sera of the proper potency are mixed

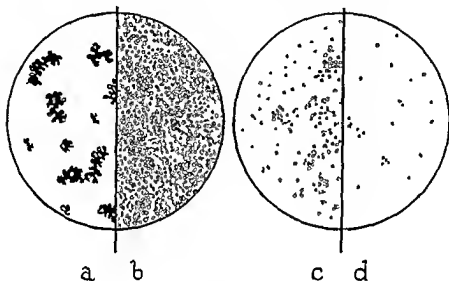


FIG. 11 DIFFERENCE IN REACTIVITY OF A AND A_2 BLOOD TO ANTI A HEMOLYSINS
(After Thomsen)

Equal parts of serum and an approximately 2 per cent suspension of blood in normal saline were mixed (a) Reaction with A_1 blood after 5 minutes and longer (b) A_2 blood after 5 minutes (c) A_1 blood after 15 minutes, (d) A_1 blood after 30 minutes. Tests were made at room temperature (18°C)

with A_1 cells the cells are lysed, but with A_2 cells, as a rule, there is distinct agglutination and little or no hemolysis (cf figure 11). Very strong sera hemolyze A_2 cells as well as A_1 cells.

That isohemolysis and isoagglutination are probably due to the same substance is supported by the observation that there is a correlation between the titer of isoagglutinin and isohemolysin, and that in absorption experiments the hemolysin and agglutinin are removed together. As a rule, isohemolysis is more frequently encountered with sera of high agglutinin titers. The absence of isohemolysis with some sera of high isoagglutinin titer and, on the other hand, its occasional presence with low titered serum, is explainable by the dependence of isohemolysis on the combined action of the isoantibodies and complement the amount

of isoantibody necessary to produce hemolysis being inversely proportional to the amount of complement in the serum

Isohemolysis is of practical importance because it may occasionally mask agglutination, and if not noticed may lead to errors in blood grouping. When sera containing potent hemolysins are used there may be inhibition of agglutination during the hemolysis (cf figure 11). This would occur only when the tests are performed with fresh serum particularly if the temperature is somewhat high. The presence of hemolysis will easily be recognized by the practiced observer. It can be avoided if the testing sera are inactivated or stored in the ice box for about a week before use. When performing tests for blood transfusions where one is pressed for time a simple way of preventing hemolysis is to dilute the serum 4 times with normal saline solution (Thomsen). The use of citrated plasma (cf page 12) instead of serum has the advantage that the citrate ion tends to inactivate the complement so that hemolysis is less frequently encountered in tests made with such plasma.

BLOOD GROUPING WITH ANIMAL SERA

Landsteiner,⁴ von Dungern and Hirschfeld^{4a} and Landsteiner and Witt⁴⁷ have shown that the sera of normal animals (such as rabbits, cattle, sheep, cats, horses, etc.) often contain agglutinins which react specifically with the human agglutinogens A and B. To demonstrate such agglutinins preliminary absorption (with group O blood for example) is usually required to remove species agglutinins acting on all human bloods. For this reason and because they are often of low titer such sera are not satisfactory for blood grouping.

Not infrequently normal guinea pig sera are encountered which contain potent hemolysins only for human A erythrocytes not accompanied by any species antibodies.⁴⁸ Of particular interest in this connection are observations⁴⁹ on the presence of group-specific agglutinins in the sera of certain species of lower monkeys.⁵⁰ In *M. rhesus* monkeys anti A agglutinins are regularly found but little or no anti B agglutinins. On the other hand the serum of the vervet monkey (*Cercopithecus nictitans*) normally contains anti B agglutinins and little or no human species agglutinins.⁵¹ Finally, in other monkeys e.g. baboon the

⁴ *Wien klin Wdsch* 1902 no 40

^{4a} *Ztschr f Immunitats* 8 526 (1910)

⁴⁷ *Jour Immunol* 11 221 (1926). With regard to the presence of anti A and anti B agglutinins in snake sera see Bond *Jour Immunol* 36 1 (1939)

⁴⁸ Cf Schiff *Die Blutgruppen und ihre Anwendungsgebiete* p. 17 (1933)

⁴⁹ Landsteiner *Jour Immunol* 15 (1928)

⁵⁰ With regard to the anthropoid apes cf page 332

⁵¹ Von Dungern and Hirschfeld *Ztschr f Immunitats* 8 526 (1911), Landsteiner

serum acts in one or the other way depending on the particular animal. An explanation for this apparently haphazard distribution of the anti A and anti B agglutinins in monkeys has recently been found by the author⁵² (cf page 335).

Landsteiner, and von Dungern and Hirschfeld showed that it is possible to produce immune sera against the agglutinogens A and B. Hence, the agglutinogens A and B are present in antigenic form in the red blood corpuscles.⁵³

Hooker and Anderson⁵⁴ who made a thorough study of this phase, reported their results in detail. These authors immunized rabbits with blood corpuscles of each of the four blood groups by several injections of blood. When the antisera were tested against bloods of each of the four groups, they agglutinated all the bloods at approximately the same titer, because of the presence in each serum of species agglutinins acting on all human blood. When the sera containing group specific agglutinins were suitably absorbed, however, agglutinins for agglutininogen A or agglutininogen B could be demonstrated.

Schiff and Adelsberger⁵ were the first to demonstrate that certain anti sheep blood immune rabbit sera agglutinate intensely human bloods of group A and group AB. This is due to the presence in group A cells of an antigen related to but not identical with the Forssman antigen present in sheep blood cells.⁵⁶ That the two antigens are not identical is shown by the fact that not all anti sheep sera exhibit this property. Just as certain anti sheep immune rabbit sera react with human A blood, so do many anti A immune rabbit sera hemolyze sheep cells (see table 65). Guinea pigs immunized with A cells on the other hand do not produce sheep cell lysins, which is explained by the presence of Forssman antigen in their organs.⁵⁷

Preparation of Anti A and Anti B Immune Sera. These reagents are prepared essentially in the manner described later for the preparation of antisera for M and N (cf page 219).

The stock sera are obtained by immunizing rabbits using group A blood when

loc cit Thomsen and Kemp *Ztschr f Immunitat* 67 251 (1930) Buchbinder *Jour Immunol* 25 33 (1933) Dahr [*Ztschr f Immunitat* 90 376 (1937)] states that the serum of *Cercopithecus fulvus* resembles macacus serum in its reactions with human blood since it contains anti A but not anti B agglutinin.

⁵² Candela, Wiener and Goss *Zoologica* 25 513 (1940) Wiener, Candela and Goss *Jour Immunol* in press.

⁵³ An antigen is defined as any substance which when injected into an animal causes the appearance in the serum of special substances—antibodies—that react specifically with the substance injected.

⁵⁴ *Jour Immunol* 6 419 (1921).

⁵⁵ *Zeitschr f Immunitat* 40 335 (1924).

⁵⁶ For a thorough review of the question of the relationship between agglutininogen A and the Forssman antigen see Andersen *Zeitschr f Rassenphysiol* 4 49 (1931).

⁵⁷ Witelsky and Okabe, *Zeitschr f Immunitat* 54 181 (1927).

preparing anti A sera group B blood for anti B sera With regard to the anti A sera some rabbits produce antisera of high titer others only weak antisera based on constitutional differences By selecting rabbits whose sera contain weak natural anti A agglutinins to begin with and who lack the group-substance A as determined by tests on the serum more uniformly successful results can be obtained (cf page 339) Wheeler Sawin and Stuart² have inbred such a strain of rabbits for the production of anti A sera Not all rabbits will produce satisfactory anti B sera and the average anti B fluid is of considerably lower titer than the average anti A serum probably because the erythrocytes of all rabbits contain an antigen related to the human B antigen (cf page 292)

Testing fluids are prepared by diluting the inactivated stock immune sera 15 to 20 times with saline solution and then absorbing with packed washed cells not containing the homologous agglutino-gen (pooled O and B blood for anti A sera pooled O and A₁ blood for anti B sera³) The absorptions usually have to be repeated several times and the author prefers to carry out the initial absorptions at room temperature the final one in the refrigerator Since the anti A and anti B agglutinins are not appreciably weakened by overabsorption it is advisable to use an excess of cells in order to avoid under absorption

The stock immune sera can be stored without any preservative in 1 c c ampoules these being placed in a water bath at 56°C for ½ hour before being labelled To the testing fluids the author adds sufficient merthiolate to give a concentration of about 1 to 10 000 Both the stock sera and the testing fluids retain their potency for many years when stored in this manner in the refrigerator

Anti A and anti B testing fluids have proved useful in studies on the properties A and B in apes and monkeys and for the examination of blood stains (cf page 414) In ordinary grouping tests the anti A testing fluids because of their high titer are useful for checking doubtful reactions

COMPLEMENT FIXATION, PRECIPITATION, HEMOTROPIC ACTION

Although, as a rule no complement fixation can be detected during isohemolysis (Schiff and Adelsberger),⁴ there are occasional human sera, containing powerful isoagglutinins and isohemolysins, which in addition give complement fixation reactions Complement fixation reactions can be more readily obtained with immune rabbit sera using as antigens saline emulsions of alcoholic extracts of red blood cells or tissues containing corresponding group specific substances⁵ with proper dilutions of the antigen, specific flocculation reactions can also be obtained with such immune sera⁶

Several authors⁷ state that normal human sera produce precipitates

² Wheeler Sawin and Stuart *Jour Immunol* 36 349 (1939)

³ Pooled blood is used in order to take care of any anti M or anti N agglutinins which may be present in the serum

⁴ *Zbl Bakter I Orig* 93 172 (1924)

⁵ Dolter *Ztschr f Immunstats* 43 95 (1925) Watebsky and Okabe *Ztschr f Immunstats* 52 359 (1927) Marberg *Ztschr f Immunstats* 80 340 (1933)

⁶ Landsteiner v d Scheer and Witt *Proc Soc Exp Biol and Med* 22 289 (1925)

⁷ Dold and Rosenberger *Klin Woch* 7 394 (1928), Ottensooser and Zurukzoglu *Klin Woch* 1932 719

with extracts of human red blood cells prepared by repeatedly freezing and warming them. These reactions, however, are probably not true precipitation, but are caused by the agglutination of stromata of the erythrocytes. On the other hand, a true precipitation of group specific substances in solution, as, for example, in serum or saliva (cf. figure 58), can be obtained with selected immune rabbit sera (Schiff)⁴⁴

It was shown by Hektoen⁴⁵ that when human erythrocytes of group A or B are mixed with certain human sera of group B or A, respectively, and fresh leucocytes are added, phagocytosis of erythrocytes will occur, a phenomenon caused by specific substances (hemotropins) in the serum. In mixtures of red blood corpuscles and sera in which antibody action is excluded, only few erythrocytes are phagocytosed. Hemotropic action is obtained especially with sera containing powerful isoagglutinins.

Two opinions have been offered concerning, in general, the various types of reactions produced with a single serum. According to one view the various phenomena, agglutination, hemolysis, precipitation, etc. are caused by different antibodies. According to the other (unitarian hypothesis), all these phenomena are produced by the same antibody, the nature of the reactions being determined only by the conditions of the experiment.

In the case of the isoantibodies and specific immune antibodies against human blood, absorption with a sufficient quantity of red blood cells will prevent all these phenomena simultaneously.

BLOOD GROUPING WITHOUT THE AID OF STANDARD SERA

The blood group of any individual can readily be determined even without the aid of standard testing sera. To illustrate the technique used, one of Landsteiner's

TABLE 6

ISOAGGLUTINATION REACTIONS OBTAINED WHEN THE BLOODS OF SIX
NORMAL INDIVIDUALS WERE TESTED WITH EACH OTHER'S SERA
(After Landsteiner)

Sera	Blood cells of					
	1	2	3	4	5	6
1 Dr St	—	+	+	+	+	—
2 Dr Plecn	—	—	+	+	—	—
3 Dr Sturl	—	+	—	—	+	—
4 Dr Erdh	—	+	—	—	+	—
5 Zar	—	—	+	+	—	—
6 Dr Landst	—	+	+	+	+	—

⁴⁴ *Klin Woch* 3 16 (1924) *ibid* 12 311 (1933)

⁴⁵ *Jour Inf Dis* 3 721 (1926) Also see Schiff *Med Klin* 21 1238 (1925)

TABLE 7

DETERMINATION OF BLOOD GROUP WITH THE AID OF BLOOD CELL
SUSPENSION AND SERUM OF GROUP A

Unknown Blood Cells Tested with Known Serum of Group A (Anti B)	Unknown Serum Tested with Known Blood Cells A	Blood Group
-	+	O
-	-	A
+	+	B
+	-	AB

TABLE 8

BLOOD GROUPING WITH TEST SERA OF GROUP O (ANTI A ANTI B) AND
GROUP A (ANTI B)
(After Schiff)

	Standard Testing Serum		Blood Group
	O αβ	A β	
Reaction with unknown blood cells	-	(-)*	O
	+	-	A
	+	+	B or AB

* Unnecessary

original experiments is given in table 6. The bloods of six normal individuals were tested by mixing the sera and blood cell suspensions in every possible combination. In this way the bloods were found to fall in three groups.⁶⁴ Bloods 1 and 6 obviously correspond to Group O since the cells are inagglutinable whereas the serum reacts with all bloods not belonging to that group. One of the pairs bloods 2 and 3 or bloods 3 and 4 must correspond to group A the other to group B. The sera of individuals of these two groups could therefore be used for testing the bloods of other individuals. The differentiation of A and B can be made by determining the distribution of the groups in a series of individuals since group A is much more frequent than group B in European races (cf. Chapter XVIII). Moreover group A is characterized by its reactions with certain anti-sheep immune rabbit sera (cf. page 31).

If a worker who has no standard testing sera available knows his own or some other individual's blood group and this person belongs to group A or group B it is possible to determine the blood group of any other individual by using the blood cell suspension and serum of the individual of known group as standard reagents. Thus if the known blood belongs to group A the four blood groups can be identified as shown in table 7. Similar reasoning holds when known group B blood is available.

If the supply of standard serum of group O is limited then as is shown in table 8 by using standard sera of group O and group A it is possible to group most bloods. Only bloods of group B and group AB cannot be differentiated from one another without the aid of standard group B testing serum.

* Blood of the relatively rare group AB was not included in the experiment.

CHAPTER III

SOURCES OF ERROR IN BLOOD GROUPING

FALSE NEGATIVE REACTIONS

IF THE testing sera are of high titer, it is practically impossible to miss positive reactions. However, although the sera may have been of high titer when put up in ampoules, the titer may fall before the serum is used. Since deterioration can occur even under sterile conditions, the contents of each vial must be tested against known cell suspensions before use. Of course contaminated sera will not be used.

When performing the tests by the slide method, a positive reaction may be missed if the mixture of cell suspension and serum is not observed for a long enough time, such as in the haste incident to an emergency blood transfusion. Furthermore, if a very concentrated blood cell suspension is used, the reactions may be considerably delayed and weakened (cf page 20). Consequently, the procedure of mixing the whole blood from the finger or ear lobe directly with the testing serum cannot be approved.

Another source of error may be low sensitivity of agglutinogens. This situation is often encountered when testing the blood of newborn infants (cf page 21) or preserved blood suspensions (cf page 20). However, even with fresh blood taken from adults, false negative reactions have been obtained, and one of the most common errors is to mistake AB blood of subgroup A₂B for group B, on account of the weak reactivity of the A agglutinin in such blood. Thus, Thomsen¹ has reported 2 cases of group AB adults whose A receptors were only demonstrable with testing sera of especially high titers.² In a case described by Polayes and Lederer³ a patient was given 18 transfusions of group B blood before it was discovered that he belonged to subgroup A₂B. Since confirmatory tests for agglutinins in the serum of infants and newborn are of little or no value, (cf page 24), in such cases it may occasionally be difficult to render an accurate diagnosis until the group has fully developed. In adults, examination of the serum will usually solve the problem, but in the instances of A₂B individuals with atypical α_1 agglutinins (cf page 204), only careful workers will succeed in making the correct diagnosis.⁴ Instances of the latter type can be rec-

¹ *Klin Woch* 8: 1075 (1929). Also see Thomsen *Ukrain Zentralbl f Blutgrup penforsch* 3: 1 (1929).

² *Laguna Klin Woch* 9: 547 (1930).

³ *Jour Amer Med Assoc* 95: 407 (1930).

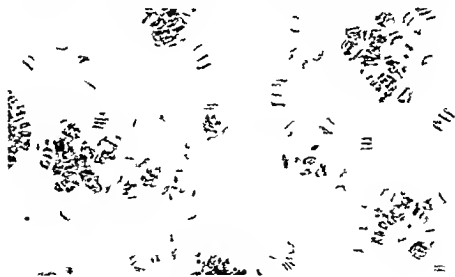
⁴ In one case an erroneous exclusion of paternity was made: the putative father

ognized by testing the patient's serum against both sorts of A cells A_1 and A_2 and by using high titered anti A sera such as the immune anti A sera (cf page 31), for testing the patient's cells. Finally, mention should again be made of the existence of very rare individuals of group A belonging to the so called subgroup A_3 whose cells react even more weakly than A B blood with anti A sera (cf page 216)

We have already stated that with fresh and highly active sera hemolysis may occur and agglutination be masked (cf page 29). Hemolysis is readily recognized as such, however, by the increased transparency of the mixture and should be considered equivalent to agglutination. Methods of preventing hemolysis have already been described (cf page 30).

FALSE POSITIVE REACTIONS

Pseudoagglutination It has been known for a long time that in acute infections the red blood cells settle very rapidly. And in 1921 a test



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FIG. 12. ROULEAUX FORMATION.
Magnified 1400.

based upon this phenomenon was introduced into clinical medicine by Fahraeus*. The tendency towards rapid sedimentation has been shown

being reported as group B the mother as group O the child as group A. In tests made by the author the man proved actually to belong to group AB (subgroup $A_1B\alpha$) the child's significantly also belonged to subgroup A_1 [Hektoen Landsteiner and Wiener *Jour Amer Med Assoc* 108: 2138 (1931)]

*And in pregnancy in malignancy and in some other conditions

**Acta med Scandinav* 55: 1 (1921) *Physiol Rev* 9: 241 (1929)

to be due to the arrangement of the red blood cells in piles like coins, a phenomenon which is known as rouleaux formation (cf fig 12) When the rouleaux are very marked, the clumps become more irregular and simulate true agglutination (pseudoagglutination)

In 1899, while examining suspensions of blood cells in the serum of patients suffering from pneumonia, Shattock⁷ observed and gave an accurate description of rouleaux formation This was subsequently confused with true agglutination, and some authors have incorrectly ascribed the discovery of isoagglutination to Shattock.⁸

A comparison of the microscopic picture of marked pseudoagglutination and true isoagglutination is given in figures 13 and 14⁹

A thorough study of pseudoagglutination has been made by Wiltshire¹⁰ The main factors which favor its occurrence are high concentration of the serum and high temperature As Shattock pointed out, pseudo-agglutination disappears on slight dilution (1 to 2 or 1 to 3) of the serum, thereby it can readily be distinguished from isoagglutination When the tests are performed in the test tube, as recommended by Landsteiner, the dilution of the serum is 1 to 3 so that pseudoagglutination hardly ever occurs However, when the tests are performed on the slide, the dilution of the serum is only 1 to 2 and rouleaux formation is favored probably because of the large area of contact between the fluid and the glass Moreover, the large surface favors evaporation and concentration of the serum The practiced observer can usually recognize pseudoagglutination by examination under the microscope (cf fig 13) The clumps resulting from false agglutination can mostly be destroyed by the addition of a drop of saline, or, as a rule, by pressing down on the cover slip over the preparation, neither of which procedures will affect true agglutination

Pseudoagglutination has been shown to be due to substances in the sick patient's serum which affect its viscosity The nature of the substances is not completely understood, but marked rouleaux formation and rapid sedimentation are closely correlated with increased fibrinogen

⁷ *Jour Pathol and Bacteriol* 6 303 (1900)

⁸ In this connection it should be pointed out that several authors have confused an observation reported by Maragliano in 1892 with isohemolysis Maragliano claimed to have observed that certain pathological sera had the power of destroying human red blood cells and hemoglobin, and that this power also extended to the patient's own blood cells This phenomenon if it really exists would be different from isohemolysis [*Verhandl d Congres f Inn Medcin* 11 152 (1892)]

With reference to the erroneous contention that isoagglutination was known in Japan and China during the 13th century see Wiener, Lederer and Polayes, *Jour Immunol* 17 357 (1929) Schiff *Deutsch med Woch* 55 1141 (1929)

⁹ Coca *Jour Immunol* 20 263 (1931)

¹⁰ *Jour Pathol and Bacteriol* 17 282 (1912)

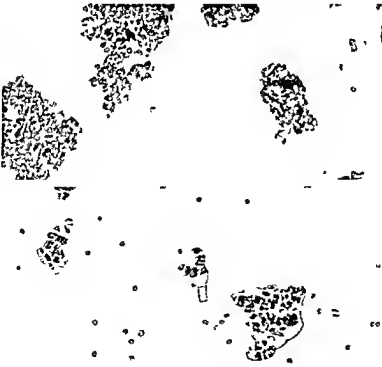


FIG 13 PSEUDOAGGLUTINATION

These two figures represent two different cases of pregnant women whose blood showed rapid sedimentation. Tests were made by mixing each serum with own cells on a slide and then covering the mixtures with a cover slip. Note that the clumps contain rouleaux (Magnified 1200)



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FIG 14 TRUE ISOAGGLUTINATION

Note that cells are tightly packed and coalescent rouleaux are absent

concentration in the plasma¹¹ and a correlation also exists with changes in some of the other serum proteins. It has been shown that the active agent is not absorbed by red blood corpuscles. True isoagglutinins, on the other hand, are absorbed by the susceptible red blood cells. Furthermore, pseudoagglutination is nonspecific, so that the serum of a patient with a rapid sedimentation, will cause rouleaux formation when mixed with any red blood cell suspension including the patient's own blood, although apparently not always to the same degree.¹²

Rarely the inequality in the capacity of different bloods to undergo pseudoagglutination may be pronounced as in a case seen by Landsteiner.¹³ The patient's blood was found to be apparently incompatible with the blood of donors of the same group. The patient's undiluted serum gave strong clumping and rouleaux with concentrated cell suspensions of these individuals in tests both on the slide and in the tube but there was little or no clumping when the serum was tested against the patient's own cells. The reaction broke up on shaking or slight dilution and was not caused by an absorbable agglutinin. A transfusion of 300 cc of blood was given without any untoward reaction.

Mention may be made of the production of phenomena resembling agglutination by means of viscous substances such as gum acacia, gum tragacanth, and gelatin. It is generally accepted that these phenomena are related to pseudoagglutination.

Autoagglutination. This phenomenon is defined as the agglutination of an individual's red cells by his own serum, due to the presence of an absorbable agglutinin in the serum and a corresponding agglutigen in the cells. While Amzel and Hirsfeld¹⁴ believed autoagglutination to be of infrequent occurrence, Landsteiner and Levine¹⁵ and Kettel¹⁶ have shown that this phenomenon is common with blood from normal individuals, in whom it occurs, however, only in tests made at low temperature.

Considerable confusion has resulted because sera containing autoagglutinins act not only on the subject's own cells, but also on the

¹¹ Fabraeus *Physiol Rev* 9: 241 (1929). Riemann *Jour Amer Med Assoc* 99: 1411 (1932). Ham and Curtis *Medicine* 17: 44 (1938).

¹² Because of this property of pseudoagglutination the phenomenon has also been designated by the names autohemagglutination, panagglutination, etc. Since the phenomenon is not produced by an absorbable agglutinin it is not a true agglutination in the serological sense so that pseudoagglutination seems to be the most appropriate name.

¹³ Personal communication.

¹⁴ *Zeitschr f Immunitalis* 43: 526 (1924).

¹⁵ Landsteiner and Levine *Jour Immunol* 12: 441 (1926). With regard to autoagglutinins in animal sera cf Landsteiner *Munch med Woch* 51: 1590 (1902).

¹⁶ Kettel *Undersogelser over Kuldehaemagglutiner i Menneskeserum* Copenhagen (1930).

TABLE 9
 AGGLUTINATION OF THE RED BLOOD CELLS OF A PATIENT (WITH RAYNAUD'S SYNDROME AND PARONYCHIAL
 HEMOGLOBINURIA) BY HIS OWN SERUM
 (After McCombs and McIlroy)

Temperature at Which Tests Were Done	Dilution of Serum										
	1 1	1 2	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024
10°C	+	+	+	+	+	+	+	+	+	+	+
15°C	+	+	+	+	+	+	+	+	+	+	+
27°C	+	+	+	+	+	+	+	+	0	0	0
37°C	+	+	0	+	0	0	0	0	0	0	0

In this patient, exposure of the extremities to cold produced symptoms of peripheral vascular nature, which remitted when the parts were warmed. The phenomenon was explained by intracapillary autoagglutination occurring *in vivo* and resulting from chilling of the blood.

bloods of all other human beings regardless of the blood group. That effectively only a single agglutinin is involved is proved by the observation that it can be completely removed by absorption with any human blood, including that of the person from whom the serum is derived. The autoagglutinins are therefore really panagglutinins,¹⁷ reacting with an agglutigen shared by all human bloods. It is best to retain the terms autoagglutinin and autoagglutination, however, in order to avoid confusion with other phenomena (pseudoagglutination, bacteriogenic agglutination) to which the ambiguous term panagglutination has also been applied.

Aside from its lack of group specificity, autoagglutination differs from isoagglutination in its great sensitivity to temperature, the titer dropping markedly as the temperature rises (cf. table 9). As a rule, the reaction is readily reversed by warming the mixture to body temperature, as by placing the tube or slide in the palm of the hand. The reaction can then be made to reappear by chilling, and this procedure can be repeated indefinitely.

From these properties of autoagglutination, it is evident that for the demonstration of the phenomenon it is best to separate the serum from the clot at body temperature. Moreover, as Landsterner¹⁸ has pointed out, the active principle can be extracted readily from the serum in a concentrated, purified form as follows. Serum containing autoagglutinins is mixed with the packed washed cells of the subject or any individual of the same group and the mixture allowed to stand in the refrigerator. The absorbing blood is separated from the serum by centrifuging in cups containing cracked ice, and the autoagglutinins are liberated from the cells by mixing the sedimented blood cells with a small amount of saline at room or body temperature. The warm saline is then separated from the cells by centrifuging, and it will be found to contain the autoagglutinin. Concomitantly with the absorption of the autoagglutinin at low temperature and its freeing at higher temperature, the cells clump and disperse again as already described.

Whereas the presence of non-specific autoagglutinins is a normal physiological phenomenon, being present also in many animal sera, it has been found that the titer of these agglutinins is considerably increased in certain pathological conditions, so that the agglutination may occur in such cases even at room temperatures.¹⁹ Some of the conditions in which this has been found are: paroxysmal hemoglobinuria (the presence of abnormal autohemolysins in this condition is the basis for the Donath-Landsteiner reaction²⁰), syphilitic or hypertrophic cirrhosis

¹⁷ Cf. Mino, *Munch med Woch* 71: 1129 (1924).

¹⁸ *Munch Med Woch* p 1812 (1903).

¹⁹ In one case recently studied by the author, autoagglutinins were found which acted in high dilutions even at body temperature [*Amer Jour. Clin Path* 12: 189 (1942)].

²⁰ *Munch med Woch* 51: 1590 (1904). The test is performed by mixing the

of the liver, hemolytic icterus, Raynaud's syndrome (table 9), trypanosomiasis of man and animals, severe anemias, and other conditions. Such cases are infrequent, since McCombs and McLeroy²⁰ could only cull reports of 38 authentic cases of the phenomenon from the medical literature. In addition to the cases reported in the literature, however, there must be many that have not been recorded. For example, at least a dozen instances of autoagglutination at room temperature have been seen by the author. Occasionally abnormally powerful autoagglutinins have been found in the absence of disease, and in some cases such agglutinins were found to be hereditary.²¹

The presence of demonstrable autoagglutinins (though weak and active only in the cold) in normal individuals raises the question as to their physiological function, if any. As for the mechanism whereby they increase in titer in the pathological conditions listed above, the explanation suggests itself that under rare circumstances when destruction of tissue or blood cells occurs within the body, autoantibodies are produced as a result of autoimmunization. For example, autoagglutinins have been observed in acute hemolytic anemias such as may occur during sulfonamide therapy,² benzene poisoning²² and the hemolytic crises of congenital hemolytic icterus. The autoantibodies found in the serum of patients with acute hemolytic anemia may therefore be the result rather than the primary cause of the disease as Dameshek²⁴ believes. Once they appear, however, the autoantibodies may aggravate the symptoms and a vicious cycle may be established.

In rabbits the production of autoagglutinins can be induced by repeated bleedings or by repeated transfusions with rabbit blood.²³ With regard to the occurrence of cold autoagglutinins in normal rabbits, cf. Wheeler.⁴

Autoagglutinins which are active at room temperature are a source of error in blood grouping. In rare instances the autoagglutinins are so powerful that it is impossible to perform a blood count except at body temperature because of spontaneous clumping of the cells, despite the high dilution of the blood in the pipette. In such cases the patient's unwashed cell suspension may exhibit agglutination regardless of the testing serum, so that such individuals may be assigned incorrectly to

patient's serum (which should be separated at 37°C) and red cells. The mixture is first kept at ice-box temperature for 5 to 10 minutes and then warmed to 37°C when hemolysis occurs.

² *Arch. Int. Med.* 59: 107 (1937). Also see the review by Rosenthal and Corten [*Folia Hematol.* 58: 64 (1937)].

² Clough and Richter, *Bull. Johns Hopkins Hosp.* 29: 86 (1918). Guthrie and Huck, *Bull. Johns Hopkins Hosp.* 34: 80 (1923). Stewart and Harvey, *Lancet* 2: 399 (1931).

²² Antopol, Applebaum and Goldman, *Jour. Amer. Med. Assoc.* 113: 483 (1939).

²³ As in a case studied by the author and reported by Gray et al., *Jour. Amer. Med. Assoc.* 114: 1330 (1940).

²⁴ Dameshek and Schwartz, *Medicine* 19: 232 (1940).

²⁵ Matsuda, *Japan Med. World* 6: 4 (1926).

²⁶ *Jour. Immunol.* 34: 409 (1938).

group AB. For this reason, whenever an "AB reaction" is obtained, it is recommended to set up control tests of the patient's cells with his own serum. Of course, a false diagnosis of group AB can always be detected by demonstrating the presence of isoagglutinins in the serum. Autoagglutination can be avoided by performing the tests at 37°C. To obtain blood cell suspensions free from autoagglutinins, the erythrocytes are washed with normal saline solution (best at 37°C).

Autoagglutination may be the cause of difficulty (though far less frequently than the pseudoagglutination) when the patient's serum (or plasma) is cross-matched against the bloods of prospective donors for the purpose of blood transfusion (cf page 61) since serum containing autoagglutinins can clump any human blood. The serum can be freed of the interfering autoagglutinins by separating it from the cells at 0°C to 5°C, at which temperature the blood cells absorb the autoagglutinins. By using serum obtained in this manner and cells washed with warm saline solution as described above, it should be possible to group and match the blood of such patients without difficulty.

As has already been mentioned, autoagglutination is ordinarily reversible at room or body temperature. However in a case recently seen by the author,²⁷ the patient's oxalated whole blood shortly after withdrawal from the body exhibited powerful spontaneous agglutination which could not be reversed by washing with warm saline at body temperature, and smooth suspensions were obtained only by washing with saline at 50°C.

Koeppelin,²⁸ who encountered two cases of autoagglutination immunized rabbits with the bloods of these patients and found that the immune sera absorbed with normal cells agglutinated the bloods of both patients but not of normal individuals. One cannot help wondering whether Koeppelin's results were due to incomplete separation of the autoagglutinins from the patient's cells, which made them more agglutinable than other blood by the immune sera.

For convenience of reference, a table given by Landsteiner has been reproduced which summarizes the characteristics of pseudoagglutination, autoagglutination, and isoagglutination (cf table 10).²⁹

Complicated instances are the occurrence of autoagglutination along with irregular isoagglutination (see below), or as observed in a recent case, serum giving both strong pseudoagglutination and autoagglutination.³⁰

Irregular or Atypical Isoagglutination. According to Landsteiner's rule

²⁷ Reisner and Kalkstein, *Amer Jour Med Sci* 203: 313 (1942).

²⁸ *Ztschr klin Med* 129: 512 (1936), *ibid* 130: 784 (1936).

²⁹ For a thorough discussion of the phenomena, pseudoagglutination and autoagglutination, see Levine, *Ukrain Zentralbl f Blutgruppenforsch* 2: 1 (1928).

³⁰ Wiener, Oremland, Hyman and Samwick, *Amer Jour Clin Path* 11: 102 (1941).

those isoagglutinins and only those are present in the serum for which the corresponding agglutinogens are absent from the cells. This rule applies only to the isoagglutinins which determine the blood groups, and there exist isoagglutinins for factors other than A and B, which, however, are only rarely present even when the corresponding agglutinogen is absent from the cells. These are known as irregular, atypical or anomalous isoagglutinins

While the occurrence of irregular isoagglutinins in normal human sera has been known for a long time, the first systematic studies were made by Landsteiner and Levine,³¹ and by Thomsen.³² The fact that among normal individuals, irregular isoagglutinins, when present at all,

TABLE 10
DIFFERENTIATION OF PSEUDOAGGLUTINATION, AUTOAGGLUTINATION
AND ISOAGGLUTINATION
(Modified after Landsteiner)

	Pseudo- agglutination	Auto agglutination	Isoagglutination
Absorption of active principle	not adsorbable	adsorbable	adsorbable
Effect of temperature on tests	not weaker, rather stronger at 37°C than at lower temperature	occurs as a rule only at low temperatures	is little affected by changes of temperature from 0°-37°C
Effect of dilution	is inactivated by slight dilution	stands considerable dilution	stands considerable dilution
Specificity	non-specific	non-specific	group specific

are weak and usually act only in the cold ("cold agglutinins"), has caused some to confuse them with the autoagglutinins, which also act best in the cold and which are often present in the same sera. Despite the close association and overlapping between the two sorts of antibodies, a distinction can often be made because typical autoagglutinins react about equally well with all human bloods, while the "cold" isoagglutinins act only or most intensely on certain bloods, and the latter can usually be separated by suitable absorption (Landsteiner and Levine). Irregular isoagglutinins in normal sera active at room temperature are less common than the "cold variety." In tests made at 15°C,

³¹ Landsteiner and Levine, *Jour Immunol* 12: 441 (1926), *Jour Immunol* 17 1 (1929)

³² Thomsen, *Ztschr f Immunstats* 57: 301 (1923)

Landsteiner and Levine detected their presence in three per cent of the sera tested, Thomsen found 32 sera with irregular isoagglutinins among 3500 examined by tests made at room temperature

As Landsteiner and Levine first pointed out, the irregular isoagglutinins in normal human sera are of four varieties (1) Those reacting with bloods of subgroup A_1 and A_B (known as α_1 or anti A agglutinins) These are probably the most common and are found among individuals of subgroups A_2 and A_2B (2) Agglutinins reacting with all group O bloods, and less intensely with bloods of subgroup A_2 known as anti O (or α_2) agglutinins and found among individuals of subgroups A_1B and A_1 , rarely also in group B (3) Agglutinins specific for blood containing agglutinin P (originally designated as extra agglutinin 1) These occur among P negative individuals of all blood groups (4) Unclassified irregular isoagglutinins

Landsteiner and Levine pointed out that there is no sharp line between the irregular isoagglutinins that act at room temperature and those which react only in the cold (cold agglutinins) This is substantiated by a study made by Kettel²³ on the relationship between the titer of cold agglutinins at 0°C and their thermal amplitude, which is determined by the highest temperature at which they still give a reaction As is shown in figure 15 the thermal amplitude is usually directly proportional to the titer of the cold agglutinin The observation that there exists a series of agglutinins varying from those giving distinct reactions at room

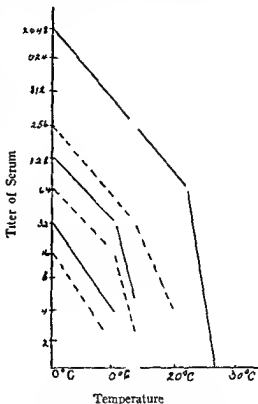


FIG 15 RELATIONSHIP BETWEEN TITER OF COLD AGGLUTININS AND THE THERMAL AMPLITUDE (After Kettel)

²³ Undersøgelser over Kuldehaemagglutinner Page 87 Copenhagen (1930)

temperature down to the ordinary cold agglutinins conforms with the theory suggested.

The presence of anomalous isoagglutinins in the serum does not in any way interfere with the determination of the group of the cells, since in all such cases the blood cells give typical reactions with the typing sera. A point of practical importance is that the atypical agglutinins only exceptionally act at 37°C, and, as a rule, the reactions are considerably weaker than typical isoagglutination. Obviously, sera containing atypical agglutinins must not be used for routine blood grouping.

It should be noted that isoagglutinins,³⁴ like other hemagglutinins³⁵ are not single substances, but can be separated into several fractions. For example, the following experiment may be cited.³⁶

Anti A serum is absorbed with 1/64 volume of red blood cells (A ₁) at 37°C			
Temperature	37°C	20°C	0°C
Titer before absorption	16	64	64-128
Titer after absorption	1	16	64

The absorption has therefore failed to remove a certain fraction of the agglutinins which acts best at low temperature, but has removed a fraction of the agglutinins that has the property of acting at higher temperatures. The isoagglutinins therefore consist of a mixture of a number of fractions which vary with respect to their thermal amplitudes. This experiment also indicates that there is really no sharp line between the cold agglutinins and the typical isoagglutinins.

Aside from the irregular isoagglutinins in normal sera described above, isoagglutinins may occur as a result of isoimmunization, as after repeated transfusions or in pregnancy (cf. page 65). The immune irregular isoagglutinins most commonly encountered give reactions corresponding to property Rh, though immune isoantibodies for P, M, A₁ and O have also been described³⁷ (cf. page 65). While many of the isoimmune sera, like the natural sera, give the strongest reactions in the cold, not infrequently sera with "warm agglutinins" (Levine) are encountered which react best at body temperature, giving little or no reactions at refrigerator temperature.³⁸

Bacteriogenic Agglutination There are phenomena unrelated to isoagglutination resulting from bacterial action, and including not only agglutination dependent on changes in the blood cells, formerly desig-

³⁴ Landsteiner and Levine, *Jour Immunol* 12: 452 (1926).

³⁵ Bialosuknia and Hirsfeld, *Compt rend Soc Biol* 89: 1361 (1923).

³⁶ Thomsen, in Steffan's *Handbuch der Blutgruppenkunde* Page 48. J. F. Lehmann Munich (1932). Also see Friedreich, *Zeitschr f Immunitat* 71: 26 (1931).

³⁷ Wiener, *Arch Path* 32: 227 (1941), Wiener, *Amer Jour Clin Path* 12: 302 (1942).

³⁸ Zacho, *Ztschr f Rassenphysiol* 8: 1 (1936), Levine, Katzin and Burnham, *Proc Soc Exp Biol and Med* 45: 346 (1940), Wiener and Toter, *Proc Soc Exp Biol and Med* 47: 215 (1941).

nated as 'panagglutination' or the 'Hubener Thomsen phenomenon,' but also reactions due to changes in the serum. Because these phenomena depend on bacterial action, Davidsohn and Toharsky³⁹ have suggested the name 'bacteriogenic agglutination'. Obviously, the phenomena will not occur when perfectly fresh blood and serum are used for grouping tests.

Bacteriogenic agglutination was first observed by Hubener⁴⁰ and Schiff and Halberstaedter⁴¹ in tests made on blood that had been kept for a number of days, and subsequently was also found to occur in tests on menstrual blood⁴² and postmortem blood.⁴³ The earlier investigators⁴¹⁻⁴³ noticed that some samples of stored blood became panagglutinable, that is, while the blood suspensions usually appeared unchanged or only slightly discolored purple, the cells were clumped by any normal human serum including that of the patient from whom the blood had been obtained. Thomsen⁴⁴ suspected that the phenomenon was of bacterial origin because he found that the property of panagglutinability could be transferred to fresh cell suspensions by the addition of a drop of the panagglutinable blood suspension, the phenomenon appearing after an incubation period of 12 to 24 hours at room temperature. Friedenreich⁴⁵ succeeded in isolating bacteria from panagglutinable blood which had the capacity of inducing the phenomenon in fresh blood. The microorganisms in question he named M and J bacilli, descriptive of their peculiar morphology, and tests on large numbers of other bacteria revealed that this property was shared by only a few of them notably members of the genus *Corynebacterium* and by certain vibrios.⁴⁶ According to Friedenreich, the bacteria produce an enzyme which acts on the red blood cells. The enzyme transforms a latent receptor in the erythrocytes into an active agglutino-gen which Friedenreich has designated as T. Anti T agglutinins are present normally in almost all human sera except those of young infants, so that such transformed blood cells usually react like group AB cells.

³⁹ Davidsohn and Toharsky *Jour Inf Dis* 67: 25 (1940) *Jour Immunol* 43: 213 (1942)

⁴⁰ *Zeitschr f Immunstats* 45: 223 (1925)

⁴¹ *Zeitschr, f Immunstats* 48: 414 (1926)

⁴² *Wiener Jour Amer Med Assoc* 97: 1245 (1931)

⁴³ Wiener unpublished observations

⁴⁴ *Zeitschr f Immunstats* 52: 85 (1927)

⁴⁵ Friedenreich *The Thomsen Hemagglutination Phenomenon* Levin and Munksgaard Copenhagen (1930)

⁴⁶ Other bacteria having this property are the T cocci of Ferada [*Taiwan Igakkai Zasshi* 35: 1267 (1936)] and Davidsohn and Toharsky's *Corynebacterium H* (Hektoenn) cultures of which can be obtained from the American Type Culture Collection Georgetown Medical School Washington D C

with the standard test sera. The pitfall in grouping can be avoided by noting that the cells are also agglutinated by group AB serum, by demonstrating the presence of anti-A or anti-B isoagglutinins in the patient's serum, or by repeating the tests on a fresh sample of blood. This serves to emphasize the advice already given to use only fresh cell suspension for blood grouping (cf. page 20). If fresh cell suspensions are not available, the possibility of the occurrence of the Thomsen phenomenon must be borne in mind. Thomsen points out that this phenomenon is rarely observed in blood that has been kept in the form of a clot.

That T agglutination is due to an antigen-antibody reaction is suggested by the fact that the anti-T agglutinin is absorbable by transformed cells but not by normal blood. Indeed, by suitable absorption tests or by using test sera from which the anti-T agglutinins have been removed by treatment with transformed group O cells, the correct group of the blood can be determined despite the transformation of the cells. In this connection, it is of interest to note that Friedenreich has shown that guinea pigs specifically sensitized by injections of transformed blood cells can be shocked by further injection of such blood but not by normal blood.

Levine and Katzin¹⁷ have recently reported a peculiar phenomenon observed with the blood of a patient 4 years of age suffering from an infection with pneumococcus I who had been treated with serum and sulfanilamide. The patient's blood belonged to group O yet it was specifically agglutinated by about 25 per cent of normal sera of all groups so that the blood cells evidently contained a special abnormal agglutinable substance. This was apparently an acquired characteristic since it could not be demonstrated in the patient's blood 4 months later. In some respects the phenomenon observed by Levine and Katzin resembles the Thomsen phenomenon except that the change occurs *in vivo*.

As pointed out above, the transformation of a latent receptor in the red cells into an active agglutino-gen T through the action of bacterial enzymes, may give rise to errors in blood grouping. A second mechanism by which bacteriogenic agglutination can occur is the production of substances capable of causing agglutination of blood cells. For example in a case reported by Grove and Crum,¹⁸ a bacterial contamination was the cause of an error in blood grouping. In this case, the bacterium in question, a mustard bacillus, had infected the testing serum which thereby acquired the capacity to agglutinate all human cells. While this phenomenon is quite distinct from T-agglutination, Davidsohn

¹⁷ *Proc. Soc. Exp. Biol. and Med.* 39: 167 (1938).

¹⁸ *Jour. Lab. and Clin. Med.* 16: 259 (1930) cf. Lacy *Jour. Immunol.* 14: 189 (1927) also cf. Kappers *Deutsch. med. Woch.* 64: 534 (1938), Eisenberg *Centralbl. f. Bakteriologie* 34: 739 (1903).

and Toharsky have shown the agglutinating substance to be likewise absorbable by susceptible cells. Infection by bacteria capable of causing bacteriogenic agglutination may produce only slight and hardly noticeable clouding of the test sera, but by the usual control tests this source of error will be detected. Bacteriogenic agglutination of the second type has been designated H agglutination by Davidsohn and Toharsky, in order to distinguish it from the Hubener Thomsen phenomenon (T agglutination), and because they observed the phenomenon in serum inoculated with *Corynebacterium H*.

According to Davidsohn and Toharsky, the formation of the bacteriogenic agglutinins results from the transformation of some constituent of the serum by a bacterial product present in filtrates of cultures. Formalin in dilutions up to 1:10,000 was found capable of preventing the formation of bacteriogenic agglutinins in sera inoculated with cultures of the bacterium as well as sera mixed with active filtrates. Merthiolate in dilutions up to 1:1,000, acriflavine, brilliant green and gentian violet in 0.1 per cent concentration also prevented the appearance of bacteriogenic agglutinins.

*Secondary Coagulation Simulating Agglutination*⁴⁹ When tests are made with unwashed cell suspensions prepared from whole blood taken directly from the finger, and with fresh serum or plasma, secondary coagulation can occur and be mistaken for agglutination. However, coagulation is readily recognized by the gross appearance of the clot and on microscopic examination. This phenomenon does not occur with washed cell suspensions or when stored or inactivated sera are used for the tests.

False Agglutination by Umbilical Cord Sera While testing a series of sera obtained from umbilical cord blood against known cell suspensions of groups A and B on the open slide, the author observed a phenomenon which resembled true agglutination. It was found with some of these sera, that when the slide was tilted back and forth, the cells came together in clumps, but these clumps broke up again if the slide was allowed to remain at rest. This process could be repeated indefinitely and occurred with any blood suspension including that of the sample from which the serum was derived. It was finally demonstrated that Wharton's jelly from the umbilical cord was responsible for the phenomenon.⁵⁰

⁴⁹ Cf. Lusena *Minerva med.* 9: 64 (1929).

⁵⁰ Polayes, Lederer and Wiener *Jour. Immunol.* 17: 345 (1929).

CHAPTER IV

HISTORY OF BLOOD TRANSFUSION

From early historical times the use of blood as a therapeutic measure was advocated some people believing that the blood not only carried the vital force of the body but was also the seat of the soul Thus Pliny and Celsus describe the custom of the people who rushed into the arena to drink the blood of dying gladiators During the middle ages the drinking of blood was much recommended for rejuvenation and the treatment of various diseases and the often cited transfusion of the blood of three youths into Pope Innocent VIII in 1492 was probably of this nature

Hieronymus Cardanus (1505 1576) and Magnus Pegelius suggested the possibility of transferring blood directly from the blood vessels of one individual into those of another and Andreas Libavius (1615) was the first definitely to advocate blood transfusion describing a technic similar to that used until quite recently Libavius wrote in 1615 Let there be a young man robust full of spirituous blood and also an old man thin emaciated his strength exhausted hardly able to retain his soul Let the performer of the operation have two silver tubes fitting into each other Let him open the artery of the young man and put it into one of the tubes fastening it in Let him immediately after open the artery of the old man and put the female tube into it and then the two tubes being joined together the hot and spirituous blood of the young man will pour into the old one as it were from a fountain of life and all of his weakness will be dispelled It is doubtful whether or not Libavius ever actually carried out the experiment that he proposed (Scheel)

The actual beginning of the history of blood transfusion should be dated from the discovery of the circulation of the blood by Harvey in 1616 and the publication in 1628 of his immortal monograph *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* This was followed in 1658 by the experiments of Christopher Wren an astronomer and architect who injected medicaments into the veins of dogs by means of slender quills fastened to bladders These experiments were continued by Robert Boyle and others and the following year the first infusion of solutions of drugs into human beings was performed in London on a convicted malefactor

The first authentic blood transfusion was performed in England by Richard Lower in 1665 In his experiments dogs which had been exsanguinated were kept alive by transfusion of blood from other dogs Lower accomplished this by connecting the carotid artery of one dog

(the donor) with the jugular vein of the other dog (the recipient) by means of quills. It is of interest to note that the technic used by Lower does not differ in any fundamental respect from one of the methods used more than 250 years later for transfusions in human beings.

The first transfusion in human beings¹ was performed in 1667, when Denys and Emmerez transfused nine ounces of blood from the carotid artery of a lamb into the vein of a young man. This transfusion was



FIG 16 CONTEMPORARY ILLUSTRATION OF EARLY LAMB BLOOD TRANSFUSION LAMSWERDE, 1684

successful, and encouraged by his success, Denys performed other similar operations. In his writings he referred to the fact that the patient passed urine as black as soot following the transfusion. In the meanwhile, Lower and King performed a successful transfusion in England, in which nine ounces of the arterial blood of a sheep was transferred to the veins of a man.

These experiments terminated when the fourth patient transfused by Denys in France died from the effects of the procedure. The symptoms exhibited by the patient who received the fatal transfusion are of interest in the light of our present knowledge. The patient, a luetic, had been transfused with some alleviation of his mania, it was claimed, twice before, the first transfusion being symptomless, but following the second transfusion "his arm became hot, the pulse rose, sweat burst out over his forehead, he complained of pain in the kid-

¹The claim that the first blood transfusion from man to man was made by Franz Folli is contradicted by Mayrhofer [*Med Welt* 12: 473 (1938)]

neys and was sick at the stomach. The next day the urine was very dark, in fact black." It is clear that the hemolytic reaction following the second transfusion was due to the immunization of the patient against sheep blood, and the third transfusion resulted in a fatal shock. A charge made by the patient's wife that her husband had been

poisoned by Denys, led to a long legal battle which ended with the exoneration of Denys, but the court decreed that further transfusions were to be prohibited, except with the sanction of the Faculty of Medicine of Paris.² Ten years later (1678) an edict of Parliament specifically prohibited the operation, thereby closing this chapter in the history of blood transfusion.

For a period of 150 years, no further progress was made and very little interest was shown in blood transfusion. In 1818, inspired by a desire to do something for the many distressing cases of death from hemorrhage occurring especially in midwifery, James Blundell attempted to revive the operation of blood transfusion. He devised a rather crude apparatus consisting of a large receptacle for the blood connected to a syringe by which the

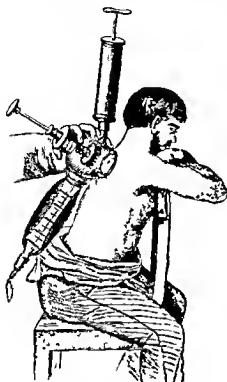


FIG 17 APPARATUS FOR CAPILLARY BLOOD TRANSFUSION DEvised BY GESELLIUS

blood was injected through a tube into the patient. From the modern point of view his technic would be less satisfactory than that used by Lower, but Blundell's researches on blood transfusion, as well as on

² For fuller information concerning the early history of blood transfusion see Scheel *Die Transfusion des Blutes*, Copenhagen (1802), Landois, *Die Transfusion des Blutes*, Leipzig (1875), Oré, *Transfusion du sang*, Paris (1876), Jennings *Transfusion*, London (1883), Roussel, *Transfusion of Human Blood* (trans from the French and German by Guinness), London (1877). For the more recent history of blood transfusion see Zimmerman and Howell *Ann Med Hist* 4: 415 (1932) [cf Correspondence, *Jour Amer Med Assoc* 100: 208 (1933)] Feinblatt *Transfusion of Blood*, New York (1926); Beck, *Erg inn Med u Kinderheilk* 30: 150 (1926), Snyder *Blood Grouping in Relation to Legal and Clinical Medicine* Baltimore (1929), Ottenberg, *Jour Mt Sinai Hosp* 4: 264 (1937), etc.

the properties of the blood and the effects of its withdrawal, served to revive the interest of the profession in blood transfusion

Through the efforts of many workers, too numerous to mention, the operation of blood transfusion was gradually perfected until today it is a safe and invaluable therapeutic procedure when performed by properly trained physicians. Before the present state of perfection could be attained, however, two main difficulties had to be overcome (1) Unfavorable reactions to transfusion caused by "incompatibility" of the bloods of the donor and recipient had to be recognized (2) Difficulties caused by coagulation of the blood had to be prevented by perfection of the transfusion apparatus and technic

INCOMPATIBILITY OF BLOOD IN RELATION TO BLOOD TRANSFUSION

Although it was noted that the transfusion of blood of domestic animals into man was often followed by hemoglobinuria (black urine), fever, or even death, animal blood was still used during the nineteenth century. In the latter half of the nineteenth century, Panum and Landois, in a series of experiments showed that while an animal which had been depleted could be saved by transfusion of the blood of another animal of the same species (as in the famous experiment of Lower), it would die if the blood of an animal of a different species was used. Landois showed that if human blood was mixed *in vitro* with the blood of other animals the human red blood cells would become hemolyzed, and the white blood cells would cease their ameboid motion and die. Similarly, Ponfick found that transfusions between animals of the same species were safe, whereas if donor and recipient of different species were used, anuria or hematuria, coma and even death might often follow.*

This work, however, did not explain why transfusions with human blood should be followed by dangerous or fatal reactions, a fact which was the cause that blood transfusion was almost completely abandoned as a therapeutic measure. These reactions were finally explained by Landsteiner in 1900,[†] when he showed that the serum of one normal human being can agglutinate or hemolyze the bloods of certain other individuals. This is undoubtedly the most important single discovery relating to blood transfusion, and Landsteiner himself,[‡] fully realizing the

* One would expect that these results would lay forever the ghost of animal blood transfusion, yet according to Zimmerman and Howell as recently as 1928 a monograph appeared in France again advocating the transfusion of animal blood into man.

[†] *Centralbl f Bakteriol* 28 357 (1900)

[‡] *Wien klin Woch* 14 1132 (1901) also see *Wiener Jour Amer Med Assoc* 100 208 (1933)

practical significance of his findings, pointed out the importance of the blood groups for blood transfusion in his original paper on blood groups. In 1907, Hektoen⁸ repeated the suggestion that the blood groups be made the basis of the selection of donors for blood transfusion. Ottenberg and Schultz⁹ were probably the first to apply Landsteiner's discovery for the determination of compatibility in an actual transfusion.

An experimental confirmation of the importance of blood grouping in transfusion was offered by the work of Ottenberg and his collaborators on transfusions in dogs and cats.⁹ In 1913, Ottenberg and Kaliski¹⁰ reported their observations on 128 human blood transfusions. In three of these cases it was shown by agglutination tests that the patient's serum reacted with the donor's cells. One of the three patients developed anuria and died eight hours after transfusion, and a second died within 48 hours. In each case blood smears made during and after the transfusions showed phagocytosis of the red blood cells. On the other hand when the bloods of donors and recipient were compatible none of these reactions occurred. Hemolytic reactions reported by other authors at about this time were also shown to be due to incompatibility.

These observations and especially the experience acquired during the first World War led to the universal adoption of blood grouping as a basis for the selection of blood donors. This has proved so satisfactory that at present there exist Blood Donor Agencies which have lists of donors of each group, so that the proper donor can be obtained on short notice.

In more recent years with the increasing popularity of blood transfusion as a therapeutic procedure, occasional instances were encountered of hemolytic reactions following the use of blood of the same group as the patient. These have been attributed to the presence of irregular isoantibodies in the patient's serum, but not in all cases were such antibodies demonstrable by cross matching tests. New information on this point was obtained in 1940 when Landsteiner and Wiener¹¹ detected a new factor in human blood (Rh) unrelated to the agglutinogens A and B, and shortly thereafter Wiener and Peters observed three instances of hemolytic reactions, one fatal in which

⁸ *Jour Amer Med Assoc* 48 1739 (1907)

⁹ *Jour Exp Med* 13 425 (1911)

¹⁰ *Berlin klin Woch* 47 1407 1437 (1910)

¹¹ Ottenberg, Kaliski and Friedman *Jour Med Research* 28 141 (1913) Ottenberg and Thalheimer *ibid* 33 213 (1915)

¹² *Jour Amer Med Assoc* 61 2138 (1913)

Proc Soc Exp Biol and Med 43 223 (1940)

¹³ *Ann Int Med* 13 2306 (1940)

isoantibodies for this factor were present in the patient's sera. These were patients who had received previous transfusions, the anti Rh isoantibodies being most likely the result of isoimmunization. Furthermore, it was found that transfusion accidents can occur at an initial transfusion due to isoimmunization in pregnancy, the fetus *in utero* supplying the foreign antigen (Levine and Stetson,¹³ Wiener and Peters,¹² Levine, Katzin and Burnham¹⁴). Some of these accidents had previously not been recognized as related to the transfusion, but were believed to be merely pregnancy complications. Apparently the great majority of the reactions in repeated transfusions and in pregnancy cases are due to the Rh factor.

DEVELOPMENT OF THE TECHNIC OF TRANSFUSION

One of the main difficulties encountered by the early workers in blood transfusion was the readiness of blood to coagulate when in contact with the apparatus or air. The coagulated blood would either clog up the apparatus, thus interfering with the continuation of the transfusion, or if introduced into the recipient's circulation might cause fatal embolism. This difficulty was combated in two ways: (A) by increasing the speed of the operation or by lining the apparatus with substances which did not favor coagulation, (B) by modifying the donor's blood so as to prevent its coagulation. The former constitutes the transfusion of unmodified blood, and the latter the transfusion of blood modified by defibrination or the addition of certain anticoagulants.

A Transfusion of Unmodified Blood. The possible methods of transfusing unmodified blood may be grouped under three headings: (1) the direct method, in which a blood vessel of the donor was sutured to a blood vessel of the recipient, (2) the semi-direct method, in which the blood was allowed to flow or was pumped through tubes from the donor to the recipient or was transferred with the aid of syringes, and (3) the indirect method, in which the donor's blood was collected in a receptacle, and then administered to the recipient by the intravenous route.

(1) It is a curious circumstance that the most difficult of the three methods of transfusion was the earliest to be perfected. Inspired by Carrel's work on end-to-end anastomosis of blood vessels, Crile¹⁵ devised a method for the direct transfusion of blood in 1907. In order to maintain the flow of blood an artery of the donor was connected to a vein of the recipient. In Crile's method a small cannula was used (cf. fig. 18) through which the vein of the recipient was drawn and cuffed back over the cannula. The artery of the donor was then drawn over the

¹³ *Jour Amer Med Assoc* 113: 126 (1939).

¹⁴ *Proc Soc Exp Biol and Med* 45: 343 (1940).

¹⁵ *Ann Surg* 46: 329 (1907).

vein, thus forming a continuous lining of intima without any rough areas to favor coagulation Crile's technic would of course have been impossible without the development of aseptic surgery at the end of the nineteenth century The direct method of transfusion has been abandoned, however, because of the following difficulties considerable surgical skill was necessary, the amount of blood transfused could not be accurately measured, an artery of the donor had to be sacrificed the same donor could only be used once or twice there was danger of



FIG 18

CRILE'S CANNULA
(Twice actual size)

transferring disease from recipient to donor, and a slight motion of either donor or recipient might sever the anastomosis, a very distressing complication

(2) By connecting the artery of the donor with the vein of the recipient by means of tubes the surgical technic can be considerably simplified This semidirect method of transfusion had been used by Lower in his experiments, already quoted, in which he connected the carotid artery of one dog to the jugular vein of a second dog by means of quills During the nineteenth century tubes made of metal, caoutchouc, pieces of artery of animals etc were used Most of the attempted transfusions were unsuccessful or only partly successful, however, since the blood would coagulate inside the tubes, and the transfusion would have to be interrupted In 1915, Bernheim¹⁶ used this method successfully by using paraffin lined tubes to connect the blood vessels of donor and recipient (cf fig 19) Since an artery of the donor must be severed, this method is open to most of the objections that have been raised against the direct method of transfusion, and it has therefore also been abandoned

For the reasons just mentioned attempts were made to transfuse blood from a vein of the donor to a vein of the recipient In this semidirect method of transfusion of blood, since there is little or no difference in pressure between the circulations of donor and recipient, external force must be used to propel the blood Blundell's apparatus was of this nature, but it was so complicated that the blood soon coagulated and only small transfusions could be given During the nineteenth century attempts were made to devise an apparatus which would speedily transfuse the blood from donor to recipient, but they were all too complicated to succeed

The present popular methods of semidirect transfusion were made possible by the use of the syringe and intravenous needle In 1892, von Ziemssen¹⁷ successfully performed a blood transfusion by drawing 20 c c of blood at a time into a syringe from the donor's vein and injecting

¹⁶ Bernheim *Blood Transfusion Hemorrhage and the Anemias* Phila (1917)

¹⁷ *Munch med Woch* 39 323 (1892)

it into that of the recipient. In 1913, Lindeman¹⁸ improved this technic by using a large number of syringes that were kept constantly clean by an assistant who washed them with saline solution. This method was not very popular because a team of trained assistants was necessary, and the danger of coagulation was ever present. Finally, in 1915, by making use of a syringe with a four way stop cock, Unger¹⁹ devised an apparatus that made possible transfusions of large measured amounts of



FIG 19 ARTERIOVENOUS TRANSFUSION BERNHEIM

blood, without placing undue hardships on either the donor or the recipient. The same year Miller²⁰ described a simple apparatus which employed a similar principle and numerous satisfactory syringe valve apparatus with different mechanisms have since been devised.

(3) The indirect transfusion of unmodified blood was made possible by the discovery that if the receptacle in which the blood is collected is evenly lined with paraffin, coagulation of the blood is delayed. A special cylinder invented by Kimpton and Brown²¹ in 1913 was used for transfusing measured amounts of unmodified blood by this method. Although the method has certain disadvantages the original Kimpton Brown tube, or modifications of the tube were still being used for blood transfusions in certain institutions as late as 1937 both in this country and abroad.

B Transfusion of Modified Blood Discouraged by his unsuccessful

¹⁸ *Amer Jour Dis Child* 6 28 (1913)

¹⁹ *Jour Amer Med Assoc* 64 582 (1915)

²⁰ *Med Rec* 88 425 (1915)

²¹ *Jour Amer Med Assoc* 61 117 (1913)

attempts to transfuse unmodified blood, Bischoff²¹ suggested defibrination of the donor's blood. For this purpose various types of apparatus were devised whereby the donor's blood could be whipped until all the fibrin had been removed. The blood was filtered through gauze to remove any small clots that may have formed and then injected into the recipient. Recently, the method has been revived, particularly by Filatov,²² who claims satisfactory results from the transfusion of conserved defibrinated blood.

The addition of phosphate of soda to the donor's blood was suggested and used by Braxton Hicks²³ as an anticoagulant, but this method was found to be dangerous on account of the harmful effect of the phosphate. Hirudin was tried at the suggestion of Landois (1892), but was discarded on account of its toxicity. Citrated blood was used for the first time by Hustin²⁴ in 1914 but his method consisted in infusing highly diluted blood mixed with sodium citrate and dextrose. At the beginning of 1915 Agote²⁵ and Lewisohn²⁷ simultaneously described the transfusion of citrated whole blood, but Lewisohn worked out the technic in detail, especially the amount of citrate to be used as an anticoagulant. Because of its simplicity, the method was used extensively during the first World War.

Recent Developments During the two decades following the first World War a controversy existed as to the relative merits of citrated blood and unmodified blood for transfusion. The proponents of unmodified blood transfusions pointed to the supposed toxic action of the citrate ion and the physiologic advantages of injecting blood in as natural a state as possible. The proponents of the citrate method, on the other hand, pointed out its simplicity, and that sodium citrate in the doses required for transfusion was harmless, while the rapidity with which transfusions of unmodified blood must be carried out to avoid coagulation can be dangerous to patients with weak myocardia. Salant and Wise²⁸ showed that while the citrate ion is stable in blood stored in vitro, it is rapidly oxidized by the tissues in vivo so that the body can tolerate fairly large amounts of citrate provided it is administered slowly. Utilizing this principle for carrying out massive transfusions by the drop method, Marriott and Kekwick²⁹ found that

²¹ *Arch f Anat Physiol u wiss Med* p 347 (1805)

²² *Beitr z Klin Chir* 164: 9 (1936) Also see Bagdasarov *Le Sang* 11: 460 (1937)

²³ *Guy's Hosp Rep* 14: 7 (1869)

²⁴ *Jour méd de Brux* 12: 436 (1914)

²⁵ *Ann de l'inst mod de clin med Buenos Aires Jan* (1915)

²⁷ *Med Rec* 87: 141 (1915) *Surg Gyn and Obst* 21: 37 (1915)

²⁸ *Jour Biol Chem* 28: 27 (1917)

²⁹ *Lancet* 1: 977 (1935)

relatively enormous doses of citrate could be given without harm to the patient. When it was found, moreover, that the incidence of chills and fever was *no higher following transfusions of fresh citrated than for fresh unmodified blood*,³⁰ the pendulum began to swing more definitely in favor of the citrate method. In more recent years the safety of the use of stored citrated blood and plasma for transfusions has been established, and the transfusion of unmodified blood has lost much of its favor, though there is still a small group of cases in which it remains the *method of choice*

* Rosenthal and Lewisohn, *Jour Amer Med Assoc* 100: 466 (1933); Wiener, Oremland, Hyman and Samwick, *Amer Jour Clin Path* 11: 102 (1941)

For a rather complete bibliography on the history of blood transfusion, see *Bull N. Y Acad Med* 15: 622 (1939) Also see Kilduffe and DeBakey's "The Blood Bank and the Technique and Therapeutics of Transfusions" Chapter I C. V Mosby Co, St. Louis (1942)

CHAPTER V

SELECTION OF DONORS SURVIVAL OF TRANSFUSED BLOOD CELLS

IN TRANSFUSION, two types of incompatibility are to be distinguished (1) The recipient's serum agglutinates or hemolyzes the prospective donor's cells. The use of such donors is likely to be followed by hemolytic reactions. (2) The prospective donor's serum agglutinates the recipient's cells, but the recipient's serum does not agglutinate the donor's cells. Such donors can usually be safely used, as was pointed out by Ottenberg.¹

Ottenberg explained this by the fact that if incompatible cells are transfused, they are acted upon by the recipient's concentrated serum as they enter the circulation and hemolysis or agglutination occurs. When incompatible serum is transfused, however, it is so diluted in the recipient's circulation that the antibodies may not injure the recipient's cells.² According to this principle, individuals of group O may be used as "universal donors" since their blood cells are not agglutinable. Furthermore, individuals of group AB may be regarded as 'universal recipients,' since they possess no isoagglutinins. Levine and Mabee³ have pointed out, however, that it may be dangerous to use as universal donors individuals of group O whose agglutinins are of high titer. For this reason, it is advisable, as a routine, to use donors of the same group except in the case of an emergency when only a group O donor is available. If universal donors are used, those possessing agglutinins of high titer should be avoided particularly for individuals in weakened condition or those with severe anemia.

According to a regulation recently included (June 26, 1941) in the Sanitary Code of New York State, only those group O individuals may be used as universal donors whose isoagglutinins have proved to be of low titer by actual titration. Witebsky, Klandshoj and Swanson⁴ have suggested that the addition to citrated group O blood of solutions of group substances A and B, in order to neutralize the isoagglutinins

¹ *Jour Exp Med* 13: 425 (1911)

² Absorption of the donor's agglutinins by the tissues of the recipient and neutralization by the recipient's own serum will also tend to prevent a reaction (cf chapter XVI)

³ *Jour Immunol* 8: 425 (1923). Also see Freeman and Whitehouse *Amer Jour Med Sci* 172: 664 (1926)

⁴ *Jour Amer Med Assoc* 116: 2654 (1941). Cf Wiener and Pennell *Amer Jour Med Sci* 204: 1 (1942)

in the blood, and these investigators have reported favorable results from this procedure

SELECTION OF THE DONOR

The recipient's blood must be typed so that a donor of the proper group can be obtained. In addition, the cell suspension of the prospective donor should be tested with the serum of the recipient, and vice-versa, as an additional check on the typing, and also to detect the presence of any irregular isoagglutinins. This test is known as "cross-matching."

When time is limited, the following tests will be found convenient (cf page 12). Suspensions are prepared by mixing 3 or 4 drops of blood in 2.3 c.c. of saline (to which may be added a small amount of 3 per cent sodium citrate). One drop of group A serum is placed on the left end of the slide and 1 drop of group B serum is placed on the right end of the slide. To each is added 1 drop of the cell suspension. After thorough mixing the slide is tilted back and forth for 3 to 5 minutes, after which time the mixtures may be covered with cover slips to facilitate reading under the microscope. The cross match is performed between the bloods of the patient and a donor of the same group as the patient. Ten drops of blood are mixed with 1 drop of a 5 per cent sodium citrate solution in a small tube, and the plasma is immediately separated by centrifugalization. (This amount of blood can be readily obtained by puncturing the finger.) One drop of the recipient's plasma is mixed with 1 drop of donor's cell suspension on one end of the slide, and 1 drop of donor's plasma is mixed with 1 drop of recipient's cell suspension on the other end of the slide. If no agglutination is seen in either mixture when examined under the low power of the microscope the donor may be used for the transfusion. With adequate technic, the tests can be completed within fifteen minutes.

It is advisable, in addition to test the patient's plasma (or serum) at once against known group A and group B cells as an additional check on the blood grouping tests, and it is usually possible to work out a scheme whereby this ancillary test can be done even in so called "emergency" transfusions (cf fig. 35).³ The confirmatory tests are recommended since they reduce the chance of making mistakes in blood grouping. However, a survey of American hospitals reveals that with few exceptions only cells and not sera are tested even in New York City, despite the fact that the New York Sanitary Code for 1930 requires that both tests be done.

Where the tests must be entrusted to individuals who have had only a short period of training, it may be safer to require a duplicate typing of the patient's blood with a different set of testing sera, and preferably also with a different technic, for instance the centrifuge technic (cf page 14).

Moreover, for reasons given below, an additional direct match by the test-tube method is advisable when dealing with patients receiving repeated transfusions⁴ and pregnancy cases. In each of two tubes are placed two drops of the patient's plasma, to one is added a drop of the donor's cell suspension, to the other a drop of the patient's cells (control to rule out non specific agglutination). Since irregular iso-antibodies from different patients differ with regard to the temperature at which they give the most intense reactions, if possible, the tests should be set up in

³ Cf Wiener, *Amer Jour Clin Path* 9: 145 (1939)

⁴ Wiener and Peters, *Ann Int Med* 13: 2306 (1940)

triplicate at body room and refrigerator temperatures. If only a single test is made this should be carried out at 37°C because most anti Rh agglutinins (the most common immune isoantibody) act best at this temperature⁷ (cf page 249). After one hour or longer the sediments are examined (cf page 248) then the tubes are gently shaken and the reactions read microscopically. If either the gross or microscopic reading is positive in the tube containing the donor's cells while the control test with the patient's cells is negative the donor should be rejected. In urgent cases the period of incubation may be shortened to 5 to 10 minutes the tubes centrifuged at low speed for 1 minute then gently shaken and the reactions read. It should be emphasized that a negative result even by the tube method of matching does not entirely exclude the possibility of intragroup incompatibility so that in special cases it may be necessary to resort to a biological test (cf page 71).

If the technic outlined above is carefully carried out, mistakes in grouping will not occur. However, in most hospitals a less elaborate technic of grouping is employed, and, in addition it is difficult to eliminate entirely errors due to the human equation, such as accidental interchange of blood samples or test sera, and errors in recording results. For these reasons occasional instances of transfusions of blood of an incompatible group still occur from time to time. To avoid clerical errors it is recommended that only one sample of blood be collected at a time, the tube being labelled by name, number and date when the blood is drawn. The purpose of the cross matching test is to supply a check on the grouping tests and to detect the infrequent instances where the sera contain irregular isoagglutinins.

Until recently there was considerable doubt as to the significance of the irregular isoagglutinins for transfusion reactions. As early as 1921, however, Unger⁸ remarked "I have occasionally noted that although the patient and donor are of the same group, when the bloods are tested one against the other a small number of unagglutinated clumps will be seen. Although ordinarily such donors are rejected occasionally urgent need forced me to transfuse patients in which this phenomenon occurred. In each case there was a sharp reaction—chills and fever—undoubtedly due to the occurrence of agglutination." Landsteiner and Levine,⁹ on the other hand, observed no reactions after transfusions to patients with irregular isoagglutinins. Moreover an analysis of hemolytic transfusion reactions by Bordley¹⁰ revealed evidence in almost every case of an error in grouping, pointing to *inter group* incompatibility as the important cause of such reactions. The apparent conflict between these observations has been resolved by

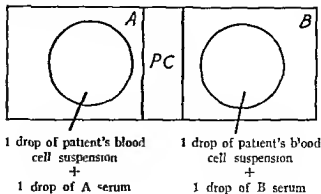
⁷ Levine, Katzin and Burnham *Proc Soc Exp Biol and Med* 45 346 (1940)
Wiener and Forer *Proc Soc Exp Biol and Med* 47 215 (1941)

⁸ *Jour Amer Med Assoc* 76 9 (1921)

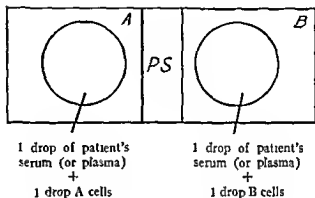
⁹ *Jour Immunol* 17 1 (1929)

¹⁰ *Arch Int Med* 47 288 (1931)

I Grouping Test on Patient's Blood Cells



II Grouping Test on Patient's Serum



III Cross Matching Test

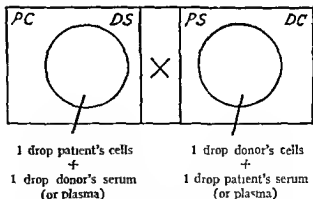


FIG 20 DIAGRAM ILLUSTRATING TECHNIC OF BLOOD GROUPING AND CROSS MATCHING BLOODS OF DONOR AND RECIPIENT, BEFORE TRANSFUSION

more recent observations which revealed that differences exist among the irregular isoagglutinins with regard to their significance for transfusion, as outlined below

The significance of *intragroup incompatibility* due to irregular iso-antibodies can best be judged by comparing this phenomenon with

intergroup incompatibility due to the isoantibodies anti A and anti B. While unintentional transfusions of blood of an incompatible group such as A to O, B to O, AB to A, etc., have caused severe or fatal hemolytic reactions, such transfusions have at times been entirely symptomless or followed only by relatively mild symptoms. For example, one instance came to the author's attention¹¹ in which a patient of group O was transfused with 1500 c.c. of group B blood from 3 different donors within a period of a few hours, without any clinical evidence of either an immediate or delayed reaction, so that this mistake was not detected until 5 days later when the patient's blood was retested before another transfusion. The reason for the absence of symptoms was probably that the patient suffered from leukemia in which disease as Davidsohn¹² has found, the isoagglutinins are ordinarily of low titer. It can be assumed that while, as a rule, incompatible blood is quickly eliminated from the circulation (within 1 or 2 days) in contrast to compatible blood which remains as a rule, for periods up to 3 or 4 months (cf. page 73) this process goes on at a slower rate when the isoantibodies are weak, thus accounting for the absence or mildness of symptoms. Transfusions of blood of an incompatible group result in an increase in the isoantibody titer by isoimmunization, because the agglutinogens A and B are antigenic in man (cf. Wiener et al.¹³). Immediately after the transfusion however the isoantibody titer is low on account of the absorption of the isoagglutinins by the incompatible blood cells (cf. figure 36). This "negative phase" lasts 2 or 3 days and then the isoagglutinin titer rises again reaching its peak (often many times the original titer) in 7 to 10 days. A second transfusion of incompatible blood at this stage will usually result in a severe or fatal reaction.

Two cases reported by Thalheimer¹⁴ and Astrowe¹⁵ illustrate the points outlined above. The patients were given transfusions of blood that was believed to be compatible and after an interval of two or three weeks the same donors were used for a second transfusion since no reaction of any sort had followed the first one. In both patients a severe hemolytic reaction occurred during the second transfusions. Astrowe's patient dying as a result of the reaction. Retests in Thalheimer's case revealed that the patient belonged to group O the donor to group B. In Astrowe's case the patient belonged to group O the donor to group A.

These observations indicate that isoantibodies due to immunization are far more dangerous than natural isoantibodies in blood trans-

¹ Wiener and Schaefer *Med Clin N Amer* 24 705 (1940)

² *Amer Jour Clin Path* 8 179 (1938) Wiener unpublished observations

³ *Amer Jour Clin Path* 11 102 (1941)

⁴ *Jour Amer Med Assoc* 76 1345 (1921)

⁵ *Jour Amer Med Assoc* 79 1511 (1922)

fusions, and this would account for the apparent discrepancy in the findings with irregular isoagglutinins. The irregular isoagglutinins studied by Landsteiner and Levine were in individuals not previously transfused (cf page 44), and transfusions of blood agglutinable by such irregular agglutinins in the serum of the patient would not as a rule, give rise to any noticeable reaction. A problem worth investigating is whether such transfusions are followed by inapparent hemolysis of the donor's blood.

In certain patients receiving repeated transfusions, on the other hand, irregular isoagglutinins may appear in the serum as a result of isoimmunization. In such patients repetition of the transfusion using either the same donor or another donor whose blood is agglutinated or lysed by the patient's serum has resulted in severe, and even fatal hemolytic reactions.¹⁶ Immune irregular isoantibodies can also result from pregnancy, in patients who have never had a previous transfusion. In such cases, the fetus in utero is the source of the foreign antigen, inherited from the father and lacking from the mother's blood (cf page 117).¹⁷ It is evident therefore that special care must be exercised in selecting donors for these two classes of patients. Particularly in such cases, but preferably as a routine, if the donor's cells are agglutinated by the patient's plasma, other donors should be tried until a completely compatible one is found. By this method it may sometimes be necessary to test a good many individuals before a suitable donor is obtained, and care must be taken not to overlook weak reactions and so to pass an unsuitable donor. A procedure to be recommended is to test patient's sera which contain irregular isoagglutinins against a series of individuals whose bloods have been examined previously for properties Rh, P and the subgroups, since the most common irregular isoagglutinins can be detected and identified in that way, and this may facilitate the selection of a suitable donor.

While the Rh factor has proved to be the most common cause of intragroup incompatibility, the writer has encountered occasional instances with immune isoagglutinins anti A,¹⁸ anti O,¹⁹ anti M and anti P.²⁰ In some cases, the irregular isoagglutinins will not correspond to any of the well classified blood properties.²¹ In rare instances, the

¹⁶ For a review of the literature cf Wiener and Peters *Ann Int Med* 13 2306 (1940)

¹⁷ Levine and Stetson *Jour Amer Med Assoc* 113 126 (1939), Wiener and Peters *loc cit*, Wiener *Arch Pathol* 32 227 (1941)

¹⁸ Wiener *Jour Immunol* 41 181 (1941)

¹⁹ Wiener, Oremland Hyman and Samwick, *Amer Jour Clin Path* 11 102 (1941)

²⁰ Wiener and Peters *loc cit*, Wiener *Amer Jour Clin Path* 12 302 (1942)

²¹ Zacho *Ztschr f Rassenphysiol* 8 1 (1936) *Veter Jour Immunol* 30 255 (1936), Levine and Polayes *Ann Int Med* 14 1903 (1941)

selection of a perfectly compatible donor may be difficult as illustrated by a case recently reported by Wiener and Forer²² The patient, who belonged to group O, had had two previous transfusions of group O blood both followed by severe hemolytic reactions Examination of her serum revealed the presence in it of two irregular isoagglutinins, anti M and anti Rh and the patient was then transfused with blood from two compatible donors, selected because they belonged to group O, type N, and were Rh negative like the patient Incidentally, less than 3 per cent of all group O donors belong to type N and are Rh negative, so that the blind selection of donors for this patient could have been a discouraging task It is of interest to note that the anti Rh agglutinin in the case just described was a warm agglutinin, while the anti M isoagglutinin was a cold agglutinin

The problem of selecting compatible donors is further complicated by the fact that intragroup incompatibility may exist even when in vitro reactions are not found This may occur if the technic of testing is not sufficiently sensitive, or if the tests are carried out too soon (negative phase) or too long a time after the immunizing transfusion and in certain cases in which the in vitro tests are continually negative despite an in vivo incompatibility, presumably due to antibodies present in tissue cells Fortunately such instances are rare Here one's only recourse may be some sort of biological test (cf page 71) For example, where the patient is Rh negative an injection of about 50 c c of Rh negative blood of the proper group can be tried—if after one to two hours no evidence of hemolysis is noted, more of the same blood can be transfused The author has followed this procedure with gratifying results in a number of cases²³

It should be pointed out that in some cases of intragroup incompatibility the in vitro tests may exhibit lysis instead of agglutination²⁴ This is a rare phenomenon and one is apt to overlook it unless one bears the possibility in mind (cf page 29)

The procedures outlined above can be carried out without too much difficulty provided that the blood transfusion is not urgently required They are not practicable, however, in obstetrical patients with post partum hemorrhage, premature separation of the placenta, etc., and in these cases the danger of intragroup incompatibility is greatest especially if the patient has given birth to an erythroblastotic infant or had a stillbirth (cf page 369) However, as already mentioned

²² *Proc Soc Exp Biol and Med* 47 215 (1941)

²³ Wiener *Arch Pathol* 32 227 (1941) Wiener Silverman and Aronson *Amer Jour Clin Path* 12 241 (1942)

²⁴ Parr and Kirschner *Jour Amer Med Assoc* 98 47 (1932) Levine and Polayes *Ann Int Med* 14 1903 (1941) Netter *Jour Immunol* 30 255 (1930)

almost all of the intragroup reactions in these cases are due to the Rh factor, so that if Rh negative donors of the same group as the patient are available, any difficulty can usually be avoided. If time permits, a direct matching should certainly be carried out in addition. In dire emergencies, on the other hand, not only the matching but the grouping of the patient's blood can be omitted, since the writer has found that group O, Rh-negative individuals with weak isoagglutinins in their sera are satisfactory universal donors for pregnancy cases, and such donors are certainly safer than donors of the same group as the patient, but selected without regard to the Rh factor.

As indicated above, in male patients who have never had previous transfusions, intragroup hemolytic transfusion reactions have not been reported. Accordingly, it is quite safe to transfuse such patients with blood of the same group without cross matching, and the author has followed this procedure in urgent cases. For this reason the idea to group all recruits and inscribe their blood group on their identification discs that is now being carried out by the U S Army and Navy is a good one.

Not only must the donor's blood be compatible with that of the recipient, but it must also be of such quality as to be likely to benefit the patient. The health of the donor must be perfect. Cases of transmission of syphilis, malaria, hypersensitiveness, measles, small pox, and other diseases from the donor to recipient have been reported. Donors giving a history of malaria should not be used, since a number of cases are known where patients developed malaria following transfusion of blood from individuals who had had the disease many years previously, and were apparently cured.²⁵ Care must also be taken to protect the donor from the transmission of disease (particularly sepsis²⁶) from the patient. By the use of the citrate method of transfusion in such cases, that possibility is excluded.

Before donors were better controlled, the men who presented themselves as donors not infrequently had low hemoglobin content, even lower than the patient. In a number of cases, the donor lent his identification book to a friend, who took the call in his place.¹ In New York City, this matter is now regulated by statute so that only licensed individuals may act as donors. Every donor is supplied with a book containing his picture and a record of his last physical examination, the hemoglobin content of his blood, the result of the Wassermann test, and each transfusion for which he donated blood.

With people of limited means the cost of the blood is of considerable importance. In the U S the cost varies between \$5 and \$10 per 100 c c of blood. A number

²⁵ *Quarterly Bulletin* Dept of Health City of N Y 4 39 (1936)

²⁶ Tzanck and Martineau *Bull Soc Med Hop de Paris* p 432 (1934)

of hospitals have at their disposal lists of volunteers members of organizations such as the American Legion police and fire departments The use of the radio or newspapers for obtaining volunteers has been resorted to in some instances Often friends or relatives of the patient are used

In London the British Red Cross organized a volunteer blood transfusion service in 1921 Donors on this service are forbidden to receive any remuneration and the identity of the patient is kept secret from them A similar blood transfusion service is operated by the Red Cross in Rotterdam The donors are not paid for their blood but are given a medal as a memento of their service (cf figure 21) The importance of these volunteer blood transfusion organizations is evident from the fact that thousands of transfusions are given every year through their assistance



FIG 21 MEDAL WITH A PICTURE OF LANOSTEIN GIVEN TO VOLUNTEER BLOOD DONORS BY THE BLOOD TRANSFUSION SERVICE OF THE RED CROSS OF ROTTERDAM

It is fairly easy to control the quality of the blood transfused when professional donors are used but when relatives or friends of the patient are used in the haste incident to the transfusion the physical examination is apt to be passed over too lightly In fact most of the cases of transmission of syphilis can be traced to the use of these so called "family" donors However now that there are simple rapid and reliable tests for syphilis like the Kline, Kahn, Mazzini tests etc at least that procedure should not be omitted before any transfusion²

Not infrequently it is found, where "family" donors must be resorted to that none of the volunteers belongs to the same group as the patient In institutions where many transfusions are performed this difficulty can be overcome by following the plan suggested by the Cook County Hospital²⁸ This plan works especially well for transfusions of citrated blood Citrated blood of all four groups is constantly kept on hand in the refrigerator Whenever a transfusion is to be given, blood of the proper group is taken from the "bank" and given to the patient In re

² Rein Weiss and Cuckerbaum *Jour Amer Med Assoc* 110 13 (1938)

²⁸ *Jour Amer Med Assoc* 109 128 (1937) *ibid* 111 317 (1938)

turn, a member of the family is expected to provide an equal amount of blood not necessarily of the same group to replace that received by the patient. This blood is subjected to a serological test, grouped, and stored for use in some future transfusion. The advantages and disadvantages of this method are discussed later (cf. page 136). To maintain the supply, blood has been drawn from patients who may be benefited by phlebotomy, such as patients with hypertension, congestive heart disease and polycythemia vera. Whether or not this is permissible from the point of view of the recipient is doubtful at least as regards the last named condition.

Usually the donation of blood by healthy individuals is not harmful to them. If the donor is too old there is danger of insufficient blood regeneration, but for the average healthy young man transfusions of 500 c c of blood every four to six weeks may be endured safely for a long time. Certain plethoric individuals can give larger quantities and there has recently been a report of a donor who gave as much as 257 liters of blood during the years 1924 to 1936.²⁹ During 1935 alone this particular donor is said to have given the astonishing total of 98 blood transfusions, averaging 300 c c each. Depending on the height and weight of a blood donor he should be able to withstand without unpleasant symptoms, the withdrawal of from 500 to 750 c c of blood. As much as 1400 c c of blood has been taken from a single individual at one time but such a procedure is not recommended. If in emergencies more than 1000 c c of blood must be taken from a donor, he should be kept in a recumbent position for an hour before being permitted to leave the operating room.

For the sake of completeness it may be mentioned that some authors have suggested the use of the blood of individuals killed in accidents³⁰ collected within 8 hours of death. However this procedure is not practicable except perhaps in time of war and at any rate the use of such blood is repugnant. Also the collection and storage of umbilical cord and retroplacental blood for use in blood transfusions has been reported.³¹

TRANSFUSIONS IN INFANTS

As pointed out on page 25 the isoagglutinins of the mother filter through the placenta into the fetus circulation, those isoagglutinins which are incompatible with the blood cells of the fetus being neutral.

²⁹ Paris Correspondent *Jour Amer Med Assoc* 106 1507 (1936). For a report of a similar case see Valter *Le Sang* 7 768 (1933).

³⁰ Judine *La transfusion de sang de cadavre a l'homme* Paris (1933). Cf. Kulkolf and Krilova *Le Sang* 9 692 (1935).

³¹ Bruskin and Farberova *Soviet Vrach Zhur* (Russian) 40 1546 (1936). Goodall, Anderson, Altman and MacPhail *Surg Gyn. and Obstet* 66 176 (1938). However see Hawkins and Brewer *Lancet* 1 132 (1939).

ized (probably by the group substances in the tissues and body fluids) and the compatible isoagglutinins persisting—their titer at birth being about one tenth the maternal titer. Within the course of a few weeks the isoagglutinins present in the newborn infant disappear, and there follows an interval of 3 to 6 months during which there are no isoagglutinins in the baby's plasma. In addition while the agglutinogens are present in the blood cells at birth the agglutination reactions of the blood of the new born are weaker as a rule than those of adults. For these reasons Unger² asserted that for infants under six months of age any donor regardless of group is compatible. He stated also that he had performed transfusions with random donors in such cases without untoward results and that it is therefore superfluous to group the infant's blood.

Some observations by Jervell³ tend to support Unger's contentions since this investigator found that in infants transfused with incompatible blood the donor's cells survived for periods beyond six weeks (cf. page 74).

Generally however it is best to group infants as well as adults and to use only donors of the homologous group. At birth as pointed out above the plasma of the infant may contain isoagglutinins of maternal origin so that the use of random donors might be dangerous. In emergencies the mother could be used as the serum of new born infants contains no agglutinins capable of acting on the mother's cells. On the other hand the indiscriminate use of maternal blood for newborn infants meets with the same objections as the random use of group O individuals as universal donors for adults and in addition maternal blood is specifically contraindicated in infants suffering from erythroblastosis foetalis (cf. page 88). Finally while in infants receiving repeated transfusions there is less danger of isoimmunization than in adults because the capacity to form antibodies is imperfectly developed in infants on the other hand isoantibodies can be transferred passively from donor to patient. For example an infant of group O receiving first a transfusion of group O blood then one of group A blood might in this way have a hemolytic reaction to the second transfusion.

Preliminary grouping and cross matching are also necessary when the transfusion is performed by the intraperitoneal route.⁴ This method of transfusion in infants has been recommended by various authors because of technical difficulties of the intravenous route in infants. Since the blood is very rapidly absorbed from the peritoneal cavity into the

² *Jour Amer Med Assoc* 76: 9 (1921)

³ *Acta Pathol et Microbiol Scand* 1: 201 (1924)

⁴ Ravenel *Jour Amer Med Assoc* 100: 473 (1933)

general circulation, this mode of administering blood may be considered as almost equivalent to a transfusion by the intravenous route, and consequently, the usual serological precautions should be taken

THE BIOLOGICAL TEST

Oehlecker³³ has recommended a test for compatibility of the bloods of a prospective donor and a patient, which serves as an additional precautionary measure

Ten to 20 cc of the donor's blood are slowly injected into the recipient's vein, and if after 10 to 20 minutes no reaction occurs, then according to Oehlecker, a larger quantity of the same blood will be tolerated. If signs of incompatibility of the blood appear (cf. page 119), however, a different donor must be used for the transfusion. Needless to say, the use of this method does not make unnecessary the preliminary serological examination and Oehlecker recommends his test only as a supplement to the usual methods of typing and cross matching of the bloods of donor and recipient.³⁴ When citrated blood is given, this test causes no inconvenience. The procedure recommended by Oehlecker however, is not entirely reliable because some patients who failed to react to the test dose had severe or even fatal reactions when the balance of the blood was transfused.

For special cases namely, patients receiving repeated transfusions postpartum cases and patients whose sera contain strong autoagglutinins the present author³⁵ has devised a new biological test which has proved useful for the prevention of intragroup hemolytic reactions.

One hundred cc. of the prospective donor's blood are mixed with 10 cc. of 3.8 per cent dihydric sodium citrate solution. 10 cc. of blood are drawn from the patient and divided between two tubes one empty and the other containing 1 cc. of the citrate solution. Through the same needle 50 cc. of the donor's blood are injected by syringe. After one hour 10 cc. of blood are again drawn from the patient and treated in the same way as the pretransfusion sample. The citrated blood samples are centrifuged at once and the color of the plasma in the two tubes compared. The serums from the clotted blood samples are later separated after the blood has clotted firmly, this serving as a check on the results of the tests with the citrated blood samples. The purpose of the duplicate test is to avoid the occurrence of an artefact due to traumatization of the red blood cells particularly when separating the clot from the wall of the tube. Naturally care must be taken that the needles and syringes used for the test are perfectly dry.

If there is no change in the appearance of the patient's plasma and the need for blood is urgent one can then proceed with the transfusion of larger amounts of blood.

³³ *Arch. klin. chir.* 116: 95 (1921).

³⁴ *Fortschr. Ther.* 7: 457 (1931). A detailed discussion of the test is given in Oehlecker, *Die Bluttransfusion* Urban & Schwarzenberg, Vienna (1933). Oehlecker, *Chirurg* 12: 533 (1940).

³⁵ Wiener, Silverman and Aronson, *Amer. Jour. Clin. Path.* 12: 241 (1942).

from the same donor. When time permits however it is preferable to inject another test dose of 50 cc. of the donor's blood and to draw a third sample of blood after an additional hour. In this way more reliable results can be obtained and the reaction in positive cases is more striking. A further refinement of the test though this is not essential is to select a donor who differs from the patient with respect to the M N type. After 100 cc. of the donor's blood have been injected in the manner outlined above it should be possible to demonstrate the survival of the donor's cells by the method of differential agglutination where the biological test is negative in patients having a red cell count of 2.5 million or less as has been pointed out by Wiener²⁷ (cf. page 73).

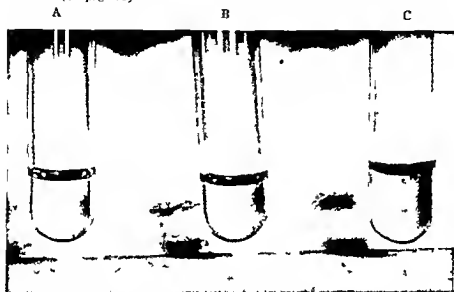


FIG. 21A. BIOLOGICAL TEST FOR INTRAGROUP INCOMPATIBILITY.

A Appearance of patient's serum before test. B Patient's serum one hour after injection of Rh negative blood. C Patient's serum one hour after the injection of Rh positive blood.

In positive reactions one hour after the test injection of 50 cc. of blood the patient may have a severe chill and rise in temperature but the clinical symptoms may be quite mild and occasionally they may be entirely absent. Therefore more reliance is to be placed on the appearance of the patient's plasma which will show a distinct rise in the icteric index as compared to the pre-transfusion sample. Inasmuch as the rise in icteric index from the hemolysis of only 50 cc. of blood is relatively small the final figure may still be within normal limits even though it is twice as high as the pre-transfusion value.

The donor's blood used for the biological test should be fresh if only bank blood is available no sample more than 3 days old should be used. The reason for this is that blood stored for periods longer than 7 to 10 days even though apparently intact when transfused often breaks down rapidly in the patient's circulation. In this

²⁷ Wiener *Amer Jour Clin Path* 12: 189 (1942)

way a positive biological reaction might be obtained even with serologically compatible blood

The appearance of a patient's serum following a negative and a positive biological test is illustrated in figure 21a. The patient in question had had hemolytic reactions following transfusions of apparently compatible blood. These reactions were explained when the patient was found to be Rh negative and her serum to contain anti Rh agglutinins (cf. page 117). The biological test furnished additional evidence that the hemolytic reactions were caused by the Rh factor.

DURATION OF LIFE OF THE TRANSFUSED BLOOD CORPUSCLE

It was believed, until very recently, that the life of the red blood corpuscle was less than 30 days. This belief was based upon estimates of the amount of bilirubin excreted daily.

By transfusion of the blood of a donor of a different group from that of the recipient, it is possible to trace the fate of the transfused red cell in the recipient's circulation, and thus obtain direct evidence as to the life of the red blood corpuscle.³³ If, for example, an individual of group A is given group O blood, the number of inagglutinable cells may be counted after the transfusion by the ordinary technic of performing a red blood count, by using a potent anti A serum instead of the usual diluting fluid. From this count must be subtracted the normal count of inagglutinable cells, which has been found to average 50 000 per cu. mm. It would be more accurate, however, when such experiments are performed, to make a count of inagglutinable cells before the transfusion as a control. As long as the count of inagglutinable cells exceeds the normal count for the patient, the presence of transfused corpuscles in the patient's circulation is assumed.

With this technic, which may be designated as the method of differential agglutination, Ashby³⁴ reported the finding of transfused blood cells in the circulation for periods up to one hundred days. Using Ashby's method, Wearn, Warren, and Ames³⁵ demonstrated that transfused red blood cells remain in circulation for periods of from 59 to 113 days, the average being 83 days.

If incompatible blood is transfused, it remains in the circulation, as a rule, only for a short time.³⁶ Therefore, even though the transfusion

³³ Cf. Plehn *Berl klin Woch* p 1862 (1914). Waldegans *Arch klin Chir* 139 1 (1926), Huck and Bigelow *Bull Johns Hopkins Hosp* 34 390 (1923).

³⁴ *Jour Exp Med* 29 267 (1919). *Arch Int Med* 34 481 (1924).

³⁵ *Arch Int Med* 29 527 (1922).

³⁶ Burnham *Arch Int Med* 46 502 (1930), Grove and Crum *Jour Lab and Clin Med* 16 259 (1930).

is tolerated, the transfusion of incompatible blood is at best of only transient benefit because of the rapid destruction of the transfused blood. However, Jervell¹² demonstrated transfused incompatible cells in an infant with melena neonatorum for more than six weeks after the transfusion. This may find its explanation in that the agglutinins of the infant probably were not fully developed (cf page 70). In a

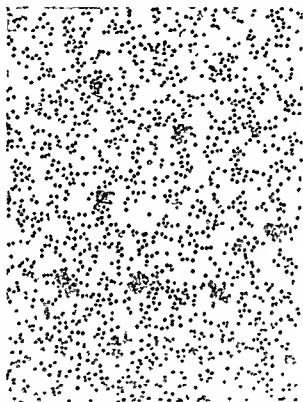


FIG. 22. AGGLUTINATION TESTS ON THE BLOOD OF A PATIENT WHO HAD BEEN TRANSFUSED WITH BLOOD OF A DIFFERENT GROUP.

Showing the reaction obtained when the red blood cell suspension of a group O infant who had received group AB blood was tested with anti A serum 2 weeks after the transfusion (cf fig 1 and fig 2).

case observed by the author, group AB blood was accidentally transfused into a group O infant six weeks of age. No reaction occurred, and the patient was followed up for 50 days, after which time he died of bronchopneumonia. Even the day before the patient died, mixtures

¹² *Acta Pathol et Microbiol Scand* 1: 201 (1924).

of the cell suspension with either group A or group B serum still presented small clumps of cells on a background of unagglutinated cells (cf fig 22)

Ashby's method has the limitation that the end point, namely, the exact time when all the donor's blood has been eliminated from the patient's circulation, cannot be determined precisely, because, as has already been mentioned, there is a certain number of inagglutinable cells in normal blood. The number of inagglutinable cells found varies with the potency of the antiserum used, and with the blood group, being higher for example, in subgroup A₂ than in subgroup A₁, particularly in newborn infants where the method may prove entirely unreliable. Moreover, Ashby's method cannot be used where transfusions of blood of the same group as the patient are given. These difficulties are overcome by testing for the agglutinogens M and N, provided that the bloods of patient and donor differ with respect to these properties. Another advantage of the latter method is that in the proper combinations the results are more striking, since, for example, if OMN blood is transfused to an ON patient, in tests made with anti M serum on the patient's blood after the transfusion only the donor's cells are agglutinated. Moreover, even in combinations such as ON to OMN (in which the unagglutinated cells in tests with anti M serum are the donor's) the transfused cells can be traced more reliably than O cells in an A individual, even in newborn infants, because very few cells of type M and type MN blood remain unagglutinated with anti M serum in infants as well as adults.

The properties M and N were first used for tracing the fate of transfused blood cells by Landsteiner, Levine and Janes,⁴³ who were able to detect the presence of donor's blood in a patient's circulation as long as 14 weeks after the transfusion. Using this technic, Wiener,⁴⁴ Martinet⁴⁵ and Dekkers⁴⁶ were able to confirm Ashby's estimates of the maximum survival time of the transfused cells as 3 to 4 months. By applying the method in a similar manner to intraperitoneal transfusions in infants, Clausen⁴⁷ found that donor's blood could be detected in the circulation within a few minutes, but he estimated that its absorption from the peritoneal cavity was not completed for periods up to 5 days. The method has been applied by the author to a comparative study of the survival time of transfused fresh and stored blood⁴⁸ (cf

⁴³ *Proc Soc Exp Biol and Med* 25 672 (1928)

⁴⁴ *Jour Amer Med Assoc* 102 1779 (1934)

⁴⁵ *Le Sang* 12 15 (1938)

⁴⁶ *Acta med scand* 99 587 (1939)

⁴⁷ *Acta Path et Microbiol Scand Supp* XXVII 134 (1938)

⁴⁸ Wiener and Schaefer *Med Clin N Amer* 24 705 (1940), Mollison and Young *Brit Med Jour* 2 797 (1941), Levine personal communication

page 137) and for the diagnosis of intragroup hemolytic reactions (cf page 124) ⁴⁸ It can also be used for estimating the circulating blood volume ^{49a}

With the aid of selected absorbed B sera that give almost complete agglutination with A blood the author has applied the subgroup differences for tracing the fate of the transfused blood cells in the combinations $A \rightleftharpoons A_1$ and $A B \rightarrow A_1 B$ (The results with combination $A_1 B \rightarrow A B$ are not reliable because of the large number of unagglutinated cells when A B blood is tested with absorbed B serum) The author has also used successfully anti Rh sera for following up transfusions of Rh negative blood to Rh positive patients and vice versa

More than one transfusion can be followed simultaneously in the same patient the maximum theoretical number with the means at our disposal being five For example if a patient of type $A_1 M N$ is given 4 successive transfusions of blood of types (1) $A M N$ (2) $O M N$ (3) $A_1 M$ and (4) $A_1 N$ then the results of these transfusions can be studied and compared with the aid of sera (1) absorbed B (2) anti A (3) anti N and (4) anti M respectively This technic has been used to compare the survival time in the same patient's circulation of blood stored for different periods of time *in vitro* ⁵⁰

In contrast to the life of the erythrocyte, that of the polymorphonuclear leucocyte is said to be very short, only 3 to 5 days ⁵¹ Very little is known of the fate of the transfused platelets, counts in thrombopenic patients indicate that transfused platelets last only 3 to 5 days

⁴⁸ Wiener and Peters *Ann Int Med* 13 2306 (1940) Wiener *Amer Jour Clin Path* 12 189 (1942)

^{49a} Todd *Proc Roy Soc B* 84 255 (1911) Ashby *Arch Int Med* 35 516 632 (1925)

⁵⁰ Wiener and Schaefer *loc cit*, Mollison and Young *Quart Jour Exp Physiol* 30 313 (1941)

⁵¹ Sabin Cunningham Doan and Kindwall *Bull Johns Hopkins Hosp* 37 14 (1925)

CHAPTER VI

INDICATIONS FOR AND RESULTS OF TRANSFUSION

THE more important indications for blood transfusion are based on the following two major effects of the procedure (1) the volume of circulating blood is increased, and (2) an immediately available supply of oxygen carrying erythrocytes is provided. By blood transfusion, the patient also receives substances essential for coagulation and the procedure is of value where this function is disturbed. In addition, transfusion supplies serum proteins, leucocytes, platelets and antibodies. The content of the other substances such as hormones, vitamins, etc., is probably too small in general to produce any significant effects, although beneficial results have been reported from transfusions in deficiency diseases.

The conditions in which blood transfusions are employed can be classified as follows

- 1 Hemorrhage
 - A Traumatic
 - B Secondary to local disease
 - C Secondary to surgical operations
- 2 Traumatic shock
- 3 Preparatory to operation
- 4 Hemorrhagic diseases
- 5 Various blood diseases
- 6 Certain poisonings
- 7 Infectious diseases
- 8 Miscellaneous conditions

Hemorrhage If more than one-third of the blood is lost in an acute hemorrhage, death usually results unless the blood is immediately replaced. In slower hemorrhages, on the other hand, a loss of two thirds of the total volume of blood may be withstood.

The most spectacular results from blood transfusion are obtained in cases of hemorrhage, which was strikingly illustrated by the saving of many lives during World War I. At Jorda's institute in Barcelona during the recent civil war in Spain, as many as 10 per cent of the casualties are said to have been saved by transfusion.¹ In civil life, most emergencies are provided by automobile accidents or cases of

¹ Jorda *Lancet* 1 773 (1939)

attempted homicide. For example, in a recent analysis² of 292 cases of gunshot wounds of the abdomen, hemorrhage was found to be the most important single factor in mortality, and a significant reduction in the mortality rate was observed among patients receiving blood transfusions.

In the sudden profound hemorrhages that occur in peptic ulcer, typhoid fever, and pulmonary tuberculosis, blood transfusion has been a life saving procedure. Cases of ulcerative colitis are also benefited by this treatment. There is no justification for the statement frequently made that transfusions should not be given in bleeding peptic ulcer lest they raise the blood pressure and thus induce more hemorrhage. This assumption is not supported by actual observation. Incidentally, in patients with normal blood pressure, the rapid transfusion of 500 c c of blood (within 5 to 10 minutes) by the syringe valve method has been found by the author to cause a rise in blood pressure of only 10 mm, while slower transfusions by the citrate-gravity method have no noticeable effect. With the almost universal adoption of the citrate method, the theoretical objection to transfusions even of large amounts of blood to patients with bleeding peptic ulcer has been removed and Marriott and Kekwick³ have obtained excellent results with continuous drip transfusions in such cases. To wait for bleeding from peptic ulcers to stop spontaneously where the hemoglobin is dropping rapidly is to invite the onset of shock from which the patient may not recover. On the other hand, because of its coagulative action, transfusion is the most effective means of bringing about the cessation of the hemorrhage barring actual ligation.⁴ It is usually necessary to repeat the transfusion several times, especially in older patients with sclerotic vessels. Where bleeding continues despite transfusion, blood transfusion may nevertheless be essential since it can make surgical treatment feasible.⁴

In obstetrical emergencies, blood transfusion has been of incalculable value. Various authors have reported successful results in postpartum hemorrhage, placenta previa, premature separation of the placenta, ruptured uterus, and ectopic gestation. The writer has often had the opportunity to observe the dramatic effect of blood transfusion in such instances.

In ectopic gestation, the blood that is recovered from the peritoneal cavity has been used for transfusion.⁵ Since such "autotransfusions

²Rippy *Jour Amer Med Assoc* 115 1760 (1940)

³*Lancet* 1 977 (1935)

⁴Polayes and Morrison *Amer Jour Med Sci* 184 326 (1932) Cf Wiener and Pennell *Amer Jour Med Sci* 204 1 (1942)

⁵Thies *Zentralbl f Gynaek* 38 1191 (1914) Schweitzer *Munch med Woch* 68 699 (1921) Rhodes and Collins *West Jour Surg* 40 252 (1932) etc.

The first recorded autotransfusion was performed successfully by John Duncan

are definitely more dangerous than the usual transfusion of fresh blood* they should only be employed in emergencies when a compatible donor is not available. The usual procedure is to collect the blood in citrate solution, and to inject the blood after filtering it through sterile gauze to remove all clots. Blood that is too old should not be used because of possible infection, or toxicity due to chemical changes. In addition to its use in ectopic gestation, autotransfusions have been employed in other instances such as hysterectomy, splenectomy, and in cases of hemothorax and ruptured liver.⁷

In cases of less profound and more prolonged or recurrent hemorrhage, the same general remarks apply as for acute hemorrhage. In secondary anemia resulting from chronic blood loss, as in menorrhagia, hemorrhoids, ulcerative colitis, epistaxis occurring particularly in hereditary telangiectasia, etc., transfusion will occasionally be required, often such patients respond well to diet and administration of adequate amounts of iron after cessation of bleeding. In hospital cases it may deserve consideration that the period of convalescence can be materially shortened by giving transfusions in place of medication.

During hemorrhage, at first, the blood pressure is sustained at a normal or even elevated level by widespread peripheral vasoconstriction. The maintenance of the normal blood pressure is aided by diffusion of fluids from the tissues into the capillaries. Finally, there is a failure of the vasoconstrictor system,⁸ the blood pressure drops and the pulse becomes feeble and rapid. When the systolic blood pressure falls below a certain minimum point, a state of shock ensues. Since, as Cutting⁹ points out, "it is generally true that the patient will not ordinarily survive depression of the systolic blood pressure below 80 mm. of mercury or of the diastolic below 40 mm. for longer than one hour," this is an indication for immediate blood transfusion.

In treating shock caused by loss of blood, speed in replacing the blood is of prime importance. In animal experiments Magladery, Solandt and Best¹⁰ found that replacement of 40 per cent of the

in 1885 [*Brit Med Jour* 1 192 (1886)] in a case of almost fatal hemorrhage occurring during the amputation of a limb.

⁷ In a series of 189 cases of ectopic gestation treated by autotransfusion Tiber [*Calif and Western Med* 41 16 (1934)] encountered 3 deaths directly attributable to the transfusion. Also see the recent review by Watson and Watson [*Amer Jour Surg* 33 232 (1936)].

⁸ White, *Surg Gyn and Obstet* 36 343 (1923), Guder, *Deutsch Zeitscher f Chir* 170 384 (1922), Brown and Debenham, *Jour Amer Assoc* 96 1223 (1931) etc. Also see Watson and Watson *loc cit*.

⁹ Blalock, *Arch Surg* 29 837 (1934). This may even occur several hours after the hemorrhage has ceased.

¹⁰ *Amer Jour Surg* 11 413 575 (1931).

^{*} *Brit Med Jour* 2 248 (1941).

blood lost was sufficient to bring about recovery but where treatment was delayed the animals failed to rally even after transfusions of quantities of blood greater than the amount lost Riddell¹ considers shock due to hemorrhage the only indication for what he terms old fashioned *rapid* blood transfusion² (in contrast to the popular citrate method) and preferably after the bleeding vessel has been ligated The ideal procedure is first to transfuse 500 to 1000 c c by a syringe valve method and then to follow this by additional transfusions by the citrate gravity method as indicated Where necessary there should be no hesitancy in transfusing as much as two liters or even more of blood In a case recently transfused by the author the axillary artery was injured during surgical removal of a lipoma from the axilla Before bleeding could be controlled about 1500 c c of blood had to be injected and the patient received a total of 2800 c c of blood during the three hours he remained in the operating room Recovery was complete and uneventful

Since satisfactory results in hemorrhage before shock sets in are obtained in 85 per cent of the cases whereas once shock sets in the number of recoveries drops to 40 per cent³ substantial hemorrhages should be treated as soon as they occur Stetson⁴ points out that too often the clinician postpones requesting blood transfusion in the hope that shock will not ensue As he states it is better to transfuse several cases that might recover without transfusion than to lose one by waiting too long Likewise MacFee and Baldrige⁵ feel that prevention of shock is much more effective than skillful treatment after shock has been established as it is easier to maintain fluid balance than to restore it

In conclusion some remarks regarding the value of blood substitutes in particular plasma or serum in the treatment of hemorrhage are in place It is generally accepted that the main danger in hemorrhage is the reduction in the patient's total blood volume which may lead to shock while the loss of erythrocytes is of less importance By restoring the blood volume in hemorrhage plasma or serum by virtue of its protein content is of great value in preventing and combatting shock (cf page 150) and produces more lasting results than saline or glucose solutions For this reason plasma transfusions are of inestimable value for the treatment of acute blood loss wherever whole blood is not readily available especially under conditions of war If blood can be

¹ Riddell *Blood Transfusion* page 143 Oxford Univ Press (1939)

² Filatov, Myanc, Kartaševsky and Doepp *Bruns Beitr Klin Chir* 161 309 (1935) Polayes and Morrison *loc cit* etc.

³ *Am J Surg* 33 537 (1936)

⁴ *Ann Surg* 91 329 (1930)

⁵ *Ann Surg* 91 329 (1930)

procured without delay then it is obviously preferable because in that way also the lost erythrocytes can be replaced. According to some authors,¹⁵ where the blood loss is very extensive, plasma appears not to be quite as well tolerated as whole blood. It has also been asserted that after plasma transfusions have been given, subsequent whole blood transfusions may help expedite convalescence.

Shock¹⁶ While the exact mechanism leading to the clinical picture of shock is not completely understood, it is agreed that the most important factor is a disproportion between the volume of circulating blood and the capacity of the vascular bed. Four types of shock are described by Harkins: (1) the hematogenic type, in which there is a loss of fluid from the blood vessels, e.g., blood in cases of hemorrhage as outlined above, or plasma in patients with severe burns and in traumatic shock, or water and electrolytes in cases of dehydration as in cholera; (2) the neurogenic type, as in fainting or following spinal anesthesia; (3) the toxicogenic or vasogenic type in histamine shock and in infections such as peritonitis; and (4) the cardiogenic type, where in contrast to the first three types with peripheral circulatory failure, the blood vessels are congested. An example of this type is the so-called "speed shock" sometimes observed after too rapid transfusions of blood.

Here we are primarily concerned with the use of transfusions of whole blood and blood substitutes in the treatment of the hematogenic type of shock. Shock may occur not only after hemorrhage but also after severe crushing injuries or excessive handling of viscera during operations, even though there is little or no visible hemorrhage. This form of shock (traumatic shock) has been attributed to injurious products of tissue autolysis which are said to affect the circulation in a manner similar to histamine, causing increased permeability and dilatation of the capillaries. Harkins believes that the transudation of plasma at the site of the injury is the chief factor. Whatever the precise mechanism, there is a definite evidence of loss of blood plasma from the vascular system as indicated by an increase in the hematocrit index. As already mentioned, where trauma is associated with hemorrhage, as is usually the case, blood transfusion is the treatment of choice. In traumatic shock with little or no hemorrhage, plasma (or serum) should be as good as or even superior to whole blood, but reliable evidence on this point is still lacking. Saline and glucose infusions are believed to be useless as these fluids quickly pass through the capillary

¹⁵ Black and Smith *Brit Med Jour* 1: 18 (1941).

¹⁶ Cf. reviews by Harkins *Surgery* 9: 231 (1941); Blalock *Principles of Surgical Care Shock and Other Problems* St. Louis (1940); Moon *Shock and Related Capillary Phenomena* New York (1938); Oxford Univ. Press.

walls and when large quantities are injected pulmonary edema may result. Solutions containing gum acacia, while superior to glucose or saline, should only be used when plasma or blood are not available, as serious reactions have been observed in a few cases.¹⁷

In severe burns, shock is said to be the most frequent cause of death and to be due mainly to the loss of plasma from the burned tissues. According to Trusler, Egbert and Williams,¹⁸ if the patient can be tided over the first 24 to 48 hours to allow the damaged capillaries to recover from the injury, the chance of recovery is greatly increased. Robertson¹⁹ has reported good results from the treatment of severe burns in children with blood transfusions, Trusler, Egbert and Williams have had good results from the use of plasma in the treatment of burn shock and point out that plasma has the advantage over whole blood that it does not produce a polycythemia. Some workers have advocated the use of exsanguination transfusions.²⁰ To prevent dehydration, the judicious administration of fluids by mouth and by vein is indicated, care being taken not to inject excessive amounts lest edema result.

In conclusion, mention should be made of the unjustified warning not to transfuse patients in shock because of the danger of overloading the circulation. When the patient is in shock, the pulse is rapid and feeble and the blood pressure low, and this may create a false impression that the heart is in a state of partial failure. Moreover, even if the patient actually has organic heart disease, in which case rapid intravenous injections might be dangerous, in the author's opinion it is still possible to perform a blood transfusion safely by the drip method (cf. page 100) unless signs of decompensation are present. By this method of transfusion which was first described by Marriott and Kelwick,²¹ the rate at which the blood is introduced into the patient's vein can be reduced to only 2 to 3 c.c. per minute so that when it becomes necessary, large quantities of blood can be administered without any danger of embarrassing the patient's circulatory system.

Preparatory to Operation. The use of blood transfusion before operation, in order to prevent shock, and to anticipate hemorrhage which may occur, has acquired an important and permanent place in surgery. Such transfusions are advisable in patients with low red blood cell counts, especially when spinal anesthesia is used, because this method of anesthesia tends to lower the blood pressure. On account of the coagulative action already mentioned, blood transfusions are of particular value for

¹⁷ Studdiford, *Surg., Gyn. and Obstet.* 64: 772 (1937).

¹⁸ *Jour. Amer. Med. Assoc.* 113: 2207 (1939). Cf. Elkinton, Wolff and Lee, *Ann. Surg.* 112: 150 (1940); Elman, *Jour. Amer. Med. Assoc.* 116: 213 (1941); Black, *Brit. Med. Jour.* 2: 693 (1940).

¹⁹ Robertson, *Jour. Canad. Med. Assoc.* 11: 744 (1921).

²⁰ The principle of this procedure is to remove the patient's blood and to replace it with normal blood (cf. page 101).

²¹ *Lancet*, p. 977, April 27 (1935).

the preparation of individuals with jaundice or hemorrhagic diseases for operative procedures Lewisohn writes, 'If both operation and transfusion be done, transfusion should preferably be done first, whether the patient be actively bleeding or not. The shock of the operation will be prevented, and a poor surgical risk is converted into a good one''

At the Jewish Hospital of Brooklyn before every brain operation and before other operations in which much bleeding is anticipated the patient is grouped and one or more compatible donors are obtained. An intravenous infusion of physiological saline solution is started before the operation and allowed to run during the operation. As needed blood is drawn from one or more of the donors and poured into the infusion flask. In order to prevent clotting in the needle saline is allowed to run slowly into the vein during the intervals when blood is not required. At times no blood at all is needed but when it is blood can be administered promptly and in the opinion of the surgeons this arrangement has been effective in producing a substantial lowering of the operative mortality.

Hemorrhagic Diseases Blood transfusion is used in hemorrhagic diseases. It has been shown that this form of therapy markedly decreases the coagulation time in hemophilia,²² so that it is a specific, although only a temporary remedy for this condition. Blood transfusion may be a life saving measure in hemorrhages occurring in hemophilia. It apparently makes little difference whether citrated or unmodified blood is transfused.²³ Since 100 c c of blood is sufficient to produce a hemostatic effect, preoperative blood transfusion may make surgical procedures possible which would otherwise be inadvisable. A hemophiliac should therefore know his own blood group so that compatible donors can quickly be obtained.

In cases of jaundice, blood transfusion is a valuable aid in the preoperative preparation of the patient, and also in the treatment of post operative hemorrhages that occur in this condition. The beneficial effect of blood transfusion in jaundice is only transient, and after three or four days the bleeding may recur. Therefore repeated small transfusions are preferable to one large transfusion.

Recently it has been found that the hemorrhagic tendency in obstructive jaundice is due to prothrombin deficiency,²⁴ and in the preoperative preparation of jaundiced patients transfusions are now replaced or supplemented by therapy with vitamin K.

Variable results have been obtained from blood transfusion in the

²² Addis *Proc Soc Exper Biol and Med* 14 19 (1916) Bulger *Jour Lab and Clin Med* 6 102 (1920) Minot and Lee *Arch Int Med* 18 474 (1916) Pemberton *Surg Gyn. and Obstet.* 28 262 (1919) etc.

²³ Lewisohn *Amer Jour Med Sci* 157 253 (1919) Ottenberg *Proc Soc Exper Biol and Med* 13 104 (1916)

²⁴ Quick Stanley Brown and Bancroft *Amer Jour Med Sci* 190 501 (1935) Cf Butt and Snell *Isotamin K* W B Saunders Co Phila (1941)

treatment of purpura hemorrhagica.²² The number of platelets is at times restored to normal for several days following the transfusion, thus permitting normal hemostasis.²³ Jagic²⁷ believes that transfusion also has a stimulating effect on the megakaryocytes in the bone-marrow with a consequent increase in the number of platelets. Since the coagulation time is normal in purpura, transfusion is not as beneficial as in hemophilia and jaundice. Engel²⁸ maintains that the erythrocytes play the important part by tiding the patient over until thrombopoiesis begins. Because splenectomy is a dangerous operation which is not always beneficial in the primary type of purpura hemorrhagica, Krasso's recommendation to try blood transfusion first is a reasonable one. In the secondary purpuras, it is of course important to find the cause and treat it.

In hemorrhagic disease of the newborn, blood transfusion has often proved to be a life-saving measure.²⁹ The beneficial effects of the transfusion depend on its hemostatic action as well as the replacement of blood. Recent investigations have revealed that during the first week of life a drop occurs in the prothrombin concentration in the blood even in normal infants, but when the prothrombin level falls too low, bleeding results. In infants with hemorrhagic disease injections of vitamin K are more effective in restoring the normal prothrombin concentration and bringing about hemostasis than injections of untyped blood by the intramuscular route, as formerly advised. Blood transfusion still plays an important rôle, however, particularly in cases where there has been much bleeding.

Various Blood Diseases. Beneficial effects following transfusion have been obtained in many blood dyscrasias.

In pernicious anemia, blood transfusion was previously the sole method of treatment, and in 1926 Yates and Thalhimer³⁰ reported a case in which a certain patient had received 113 transfusions over a period of three years. Since the introduction of liver in the treatment of this disease, blood transfusion has lost much of its value but is still used as an adjuvant, particularly during exacerbations. In-

²² Ottenberg and Libman, *Amer Jour Med Sci* 150: 36 (1915), Peterson, *Jour Amer Med Assoc* 66: 1291 (1916), Larrabee, *Jour Amer Med Assoc* 80: 838 (1923), Feinblatt, *Transfusion of Blood*, p. 61, New York (1926), etc.

²³ Krasso, *Wien Arch inn Med* 14: 377 (1927).

²⁷ *Wien klin Woch* 40: 1565 (1927), *ibid* No. 31 (1932).

²⁸ *Med Klin* 24: 888 (1928).

²⁹ Lewisohn, *Amer Jour Obstet* 77: 933 (1918), Lindquist, *Zentralbl f Gynäk* 52: 961 (1928).

³⁰ *Jour Amer Med Assoc* 87: 2156 (1926). A similar case in which a single patient was given 73 transfusions was reported by Jones, *Jour Amer Med Assoc* 85: 1673 (1926).

cidentally, the impression that blood transfusion has a stimulating effect on the bone marrow may have arisen from observations in pernicious anemia, where transfusions have brought about temporary remissions. However, such stimulating effects on the bone marrow hardly occur in other anemias.³¹

In the acute anemia described by Lederer,³² which he believes is of infectious origin, blood transfusion therapy is said to be specific.

In the disease formerly known as the hemolytic anemia of pregnancy, but renamed the macrocytic anemia of pregnancy because it has proved to be related to pernicious anemia, blood transfusions may be required for patients whose response to liver therapy is inadequate or slow. Riddell³³ states that no patient should be permitted to go into labor with a hemoglobin less than 40 per cent. If the child is to be delivered by Caesarean operation, Riddell advises that a transfusion be given three days before the delivery and another after the operation.

In aplastic anemia following pernicious anemia, or as a result of benzol poisoning or excessive roentgen ray therapy, amelioration may result from blood transfusion. Polayes and Lederer³⁴ reported a case of aplastic anemia after x-ray treatment and radium therapy for mediastinal malignancy in a man 64 years of age, in whom life was prolonged for three years by 43 blood transfusions. In Hurst's case³ the patient received 290 transfusions and was able to lead an active life for 9 years after the onset of the disease. In aplastic anemia, either idiopathic or due to poisons such as benzene, the prognosis should not be considered hopeless, especially since the marrow may resume its normal blood forming function after the patient has been carried along for a time with the aid of blood transfusions.³⁵ For example, Boon³⁷ has reported a case of aplastic anemia with recovery after 25 transfusions totalling 12,540 c.c. of blood.

Other cases of anemia occurring in the course of chronic debilitating diseases, which do not respond to treatment with liver and iron, may also supply occasion for transfusion therapy.

On the other hand, the results of blood transfusions in leukemia have been very unsatisfactory. In early cases there may be a temporary

³¹ In animal experiments it has been found that bleedings stimulate erythropoiesis whilst transfusions diminish it [cf. Whitby and Britton *Disorders of the Blood* p. 8 P. Blakiston's Son & Co (1935)].

³² *Amer Jour Med Sci* 170: 500 (1925) *ibid* 179: 228 (1930); Gordan and Blum *Amer Jour Med Sci* 194: 311 (1937).

³³ *Blood Transfusion* Oxford Univ Press (1939).

³⁴ *Jour Amer Med Assoc* 95: 407 (1930).

³⁵ Reported by Kark *Guy's Hosp Rep* 87: 243 (1937).

³⁶ Cf. Bock *New Eng Jour Med* 215: 421 (1936).

³⁷ *Brit Med Jour* 2: 104 (1938).

beneficial effect, which can be traced to improvement in the anemia. However, in severe acute leukemia blood destruction continues despite blood transfusion, and in fact, in some instances, transfusion has given rise to severe reactions, hastening the patient's death. Probably for this reason Jagic³⁸ and others consider leukemia a contra indication to blood transfusion.

Transfusion has proved to be of little value in Hodgkin's disease.³⁹ Bock⁴⁰ states that the anemia of Hodgkin's disease is not an indication for transfusion, since the presence of anemia is evidence of a terminal state. However, in early cases which are receiving radio therapy supportive transfusions to compensate for the destructive action of x rays on the bone marrow may permit more intensive treatment, and prolong the life of the patient.

In hemolytic icterus, while splenectomy is the treatment of choice when the disease is associated with severe anemia the danger of the operation is diminished by transfusion. According to Dawson⁴¹ in this disease severe transfusion reactions can occasionally occur even with perfectly matched blood.⁴² Riddell asserts, however, that the incidence of reactions in this disease is no higher than in any other anemia and the reactions observed may be due in part to isoimmunization^{43,44} and so might be avoided by the selection of appropriate donors (cf page 117).

In agranulocytosis, transfusion is said to be of little or no value.^{45,46} These poor results are easy to understand in view of the brief life of the leucocyte. Strumia⁴⁷ has proposed the transfusion of leucocyte cream, obtained by removing the white cell layer in sedimented bank blood, and other writers have suggested inducing a leucocytosis in the donors used for the transfusions. The suggestion to use leukemic blood⁴⁸ for the transfusion to patients with agranulocytosis requires no comment.

Good results have been obtained in the anemias of infancy and childhood. Since relatively small quantities of blood produce the same effect in children as much larger doses in adults, adequate treatment is more

³⁸ *Wien klin Woch* 40 1565 (1927) 45 957 (1932)

³⁹ Blumberger *Munch med Woch* 82 2023 (1935)

⁴⁰ *New Eng Jour Med* 215 421 (1936)

⁴¹ *Brit Med Jour* 1 921 963 (1931)

⁴² Sharpe and Davis *Jour Amer Med Assoc* 110 2033 (1932)

⁴³ Wiener and Peters *Ann Int Med* 13 2306 (1940)

⁴⁴ Wiener *Arch Path* 32 227 (1941)

⁴⁵ Blumberger *Munch med Woch* 82 2023 (1935)

⁴⁶ Cooksey *Jour Mich State Med Soc* 36 763 (1937)

⁴⁷ *Jour Amer Med Assoc* 187 527 (1934)

⁴⁸ Degelmann *Deutsch med Woch* p 1694 (Nov 5) 1937

likely to be given. Frequently, therefore, striking results have been obtained. In one case reported by Knauer,⁴⁹ for example, a child with 18 per cent hemoglobin and a red count of 980,000 per cu mm was rapidly revived by blood transfusion. Complete cure was effected when it was discovered that the patient was suffering from *dibothriocephalus latus* infestation. Transfusion is rarely indicated in nutritional anemia and proper diet and iron therapy usually suffice.

Patients suffering from von Jaksch's anemia recover, as a rule, when treated by transfusion, and temporary beneficial results have been obtained in Banti's disease. In a recently reported case of Winkel's disease, the patient recovered when treated with repeated blood transfusions.⁵⁰

Varying results have been reported in the treatment of sickle cell anemia with transfusions. Such a case recently seen by the writer is of interest.¹ The patient, a female, white Sicilian child, had received transfusions of a total of more than 1000 cc of apparently compatible blood (more than her blood volume) yet her hemoglobin dropped from 50 to 27 per cent. Because of her desperate clinical condition as a result of hemolysis splenectomy was considered. However despite the absence of detectable irregular isoantibodies in the patient's serum the possibility of isoimmunization was considered (cf page 117), especially since the patient had had reactions following the transfusions. Accordingly, transfusions totalling 400 cc of blood from two Rh negative donors were given, and these resulted in a prompt and lasting rise in hemoglobin to 70 per cent, as well as recovery from the clinical symptoms. The observations in this and other cases suggest that the erratic results reported in this disease and other blood diseases such as hemolytic icterus and pernicious anemia,⁵² where repeated blood transfusions are required, may sometimes be caused by isoimmunization.⁵¹

In erythroblastosis foetalis (*icterus gravis neonatorum*, fetal hydrops and *anemia hemolytica neonatorum*), hemolysis of the infant's blood cells results from the action of isoantibodies derived from the mother by filtration through the placenta (cf page 369). In some infants the disease is so severe that death occurs before treatment can be instituted, or the infant may even be stillborn. If treatment can be instituted in time and the infant is tided over by blood transfusion until the maternal isoantibodies have been eliminated from its circula-

⁴⁹ Knauer, *Die Bluttransfusion im Kindesalter* Arch. Kinderheilk. Beiheft 7 (1936).

⁵⁰ Polayes and Kramer *Jour. Ped.* 2: 482 (1933).

⁵¹ Wiener *Arch. Path.* 32: 227 (1941).

⁵² Bowcock *Johns Hopk. Hosp. Bull.* 32: 83 (1921).

tion, complete recovery may result. Maternal blood must not be injected into the infant by any route as it contains the harmful isoantibodies, and, in addition, it seems reasonable to suspect that nursing may be harmful as antibodies can also be transmitted through the milk, especially the colostrum. For transfusion blood sensitive to the isoantibodies, especially the father's blood should be avoided. Since as Levine has shown,⁵³ the great majority of the cases of erythroblastosis are due to the action of anti Rh isoantibodies. Rh negative blood of the same group as the infant is the most suitable choice as a rule.^{53a}

Acute Poisoning In cases of poisoning in which the erythrocytes have been damaged so that they can no longer function as oxygen carriers blood transfusion is useful. Carbon monoxide poisoning has been successfully treated by exsanguination transfusion. Hindse Nielsen⁴ reports recovery, following an exsanguination transfusion performed on an apparently hopeless case of nitrobenzene poisoning in a girl of 19 years. The nitrobenzene acted by changing the hemoglobin to methemoglobin and the blood transfusion saved the patient's life by supplying fresh erythrocytes. The present writer has recently seen a patient with severe hemolytic anemia which had developed during the treatment of acne with a gold compound. After repeated blood transfusion complete recovery ensued. The hemolytic anemia which develops, at times during therapy with sulfanilamide or sulfapyridine also responds well to blood transfusions.

Infectious Diseases With respect to the effects of transfusions in infectious diseases, opinions are divided. Many authors have failed to note any beneficial effect, whereas others have observed good results not entirely due, in their opinion, to the improvement of an accompanying anemia. Favorable results have been reported for example in puerperal sepsis,⁵⁴ pneumonia,⁵⁵ empyema, sinus thrombosis undulant fever etc. Recently Lantin and Guerrero⁵⁶, noted a marked reduction (47 to 24 per cent) in the mortality in severe cases of typhoid fever by treatment with exsanguination transfusion, and Seckel⁵ considers trans

⁵³ Levine, Katzin and Burnham *Jour Amer Med Assoc* 116: 825 (1941)

^{53a} When Rh negative donors are not available it has been suggested that the mother's citrated blood be centrifuged, the supernatant plasma removed and the sedimented erythrocytes transfused after resuspension in saline solution.

⁵⁴ Ugeskr. f. Læger 82: 1157 (1920)

⁵⁵ Dalsace *Bull. soc. d'obstet. et de gynec.* 21: 382 (1932). Erbsloh *Monatschr. f. Geburts. u. Gynäk.* 101: 156 (1936), etc.

⁵⁶ Wilbert *Jour Amer Med Assoc* 89: 861 (1927). Flinn *Amer Jour of Dis Child* 37: 596 (1929), Arena *Amer Jour of Dis Child* 54: 23 (1937) etc.

⁵ Quevli and Nelson *Northwest Med* 31: 12 (1932)

⁵ *Amer Jour of Med Sci* 191: 850 (1936)

⁵ *Med Klin* 31: 1603 (1935)

fusion a valuable therapeutic measure auxiliary to serotherapy in malignant diphtheria

When using transfusion therapy in infections, the main purpose is to transfuse antibacterial agents such as leucocytes, natural antibodies, complement etc., from donor to patient. Attempts are sometimes made, however, to increase this bactericidal power of the donor's blood by inducing a leucocytosis. MacLean⁸⁰ injects 2-3 c.c. of nuclein (10 per cent nucleic acid) intramuscularly into the donor and draws the blood after 2 hours, while Crocker, Valentine and Brody⁸¹ have reported good results with blood from donors injected intravenously with typhoid vaccine several hours before the transfusion.

Transfusion therapy has also been applied as a means of transferring specific immunity. In such so-called "immunotransfusions," blood from artificially immunized donors or from persons recovered from the disease in question is employed. This would seem to be a rational procedure from a theoretical point of view. In the hands of some, immunotransfusion has yielded encouraging results in the treatment of acute and chronic sepsis,⁸² in acute septic scarlet fever,⁸³ severe cases of measles⁸⁴ dysentery,⁸⁵ undulant fever,⁸⁶ etc. In subacute bacterial endocarditis, on the other hand, the method has been of no avail even when donors actively immunized against bacteria isolated from the patient's own blood stream were used.⁸⁷

Lyons⁸⁸ has recently reported favorable results in severe infections with hemolytic streptococci. Normal donors were selected who were found by appropriate tests (opsonic index) to have a relatively high titer of antibodies for the particular strain of streptococcus causing the infection. According to this author, in this way passive immunity can be conferred, sufficient to abort the infection within a few hours.

Related to the subject under discussion is the use of serum from human beings (convalescent or after recovery) instead of immune animal sera in a number of infectious diseases, such as measles and scarlet fever.

⁸⁰ Cited after Riddell *Blood Transfusion* Oxford Univ. Press (1939)

⁸¹ *Jour. Lab. and Clin. Med.* 20: 482 (1932)

⁸² Fry *Brit. Med. Jour.* 1: 290 (1920) Stetson *Amer. Jour. Med. Sci.* 168: 534 (1924) Stephenson *Jour. Amer. Med. Assoc.* 100: 100 (1933) etc.

⁸³ Gordon, *Jour. Amer. Med. Assoc.* 100: 102 (1933)

⁸⁴ Ribadeau Dumas and Bressaud *Bull. et mem. Soc. med. d. hop. de Paris* 42: 14 (1918)

⁸⁵ Turell *Jour. Lab. and Clin. Med.* 25: 706 (1940) Felsen *Amer. Jour. Dig. Dis.* 7: 81 (1940)

⁸⁶ Creswell and Wallace *Jour. Amer. Med. Assoc.* 106: 1384 (1936)

⁸⁷ Kilgore *Amer. Heart Jour.* 13: 619 (1937)

⁸⁸ *Jour. Amer. Med. Assoc.* 105: 1972 (1935)

Ramos and Montes⁶⁹ have reported the successful treatment of puerperal sepsis by transfusion of citrated blood to which arsphenamine was added after failure of therapy with sulfanilamide and immuno-transfusion. With regard to the treatment of infections by autotransfusions of patient's blood after irradiation in vitro with ultraviolet light the claims made by Miley⁷⁰ seem extravagant to the author.

In conclusion it may be mentioned that the indications for immuno-transfusions appear to have been reduced by the introduction of sulfonamide drugs in the therapy of infections. In the occasional cases of hemolysis produced by these drugs, transfusions may be required. *Miscellaneous Conditions* It would lead us too far afield to make a complete list of all the conditions in which blood transfusions have been applied, embracing such divergent conditions as epidemic encephalitis, generalized dermatitis (particularly, arsphenamine dermatitis)⁷¹ bronchial asthma, influenza, eclampsia, etc.

Favorable effects have been noted in premature infants with anemia and in nutritional disorders of infants (athrepsia and coeliac disease). In infants suffering from anhydremia with intoxication, transfusion aids the retention of body fluids, when used in conjunction with the administration of normal saline and glucose solutions.

Feinblatt and Sherman⁷² report a case of diabetic coma in which no definite improvement followed large doses of insulin, but which was immediately relieved by blood transfusion. Cornils⁷³ has reported recovery following transfusion in a case of acute yellow atrophy where death was expected.

In toxemias due to pregnancy, the technic of plasmapheresis is sometimes employed.⁷⁴ The patient is bled, the red blood corpuscles separated from the plasma, and washed in saline solution. Then they are restored to the body in saline or Locke's solution.

In anemic patients with chronic nephritis with hypertension transfusion by the syringe value method (cf. page 105) is contra-indicated as the rapid injection of blood might cause a further rise in blood pressure and possibly a cerebral hemorrhage. The author transfuses such patients by the drip method and during the infusion withdraws some of the patient's own blood from the opposite arm (which on account of the anemia entails only an inconsiderable loss of red

⁶⁹ *Prensa med. argent.* 25: 2419 (1939).

⁷⁰ *Hahnemannian Weekly* (Dec. 1940). Cf. Barrett *Med. Clin. N. Amer.* 24: 723 (1940).

⁷¹ *Kuske-Schuerer med. Woch.* 16: 1025 (1935).

⁷² *Jour. Lab. and Clin. Med.* 11: 63 (1925). Cf. Wortis and Lambert *Amer. Jour. Psychiat.* 96: 335 (1939).

⁷³ *Mitt. Grenzgebiete med. u. chir.* 38: 313 (1925).

⁷⁴ *Bock New Eng. Jour. Med.* 215: 421 (1936), cf. O'Hare, Brittingham and Drinker, *Arch. Int. Med.* 23: 304 (1919).

cells) to avoid overloading of the cardiovascular system. By this technic transfusions can be given in the presence of malignant hypertension or even in serious cardiac disease such as coronary occlusion, and by improving the complicating anemia may help to ameliorate the patient's symptoms.

*Use and Abuse of Transfusion*¹⁵ Since blood transfusion therapy entails a certain though only a slight risk, it must not be employed without a definite indication. Unfortunately, this principle is not always followed. Patients are being transfused because of general debility due to various causes, chronic nephritis, malignant hypertension, etc., with results not worth the effort or even detrimental. In cases of acute leukemia or carcinomatosis the family should be informed of the inevitable outcome rather than be subjected to the expense of a therapy which can at best prolong a miserable existence for a short time.

On the other hand, where transfusions are needed they should be given without delay and in adequate dosage. When it is realized that a transfusion of 500 c c of blood in an adult can at most produce a rise in hemoglobin of only 8 to 12 per cent, it will be seen that it is advisable to give more than 500 c c when preparing a patient with very low hemoglobin for operation. Yet, not infrequently, the same arbitrary amount is given indiscriminately whether the hemoglobin percentage is 20 or 70. Probably, the fault is not so much that the number of blood transfusions is too great, but rather that the method is not properly applied or the indications not well observed.

¹⁵ Cf. Polayes and Morrison *Amer Jour Med Sci* 184: 326 (1932). In this valuable paper the results of 1500 transfusions are critically examined. Also see Bock *loc cit*.

CHAPTER VII

TECHNIC OF TRANSFUSION OF FRESH BLOOD

IN TRANSFUSIONS, the blood is introduced into the patient's circulation by the intravenous route. The part of the technic of blood transfusion requiring most attention is the proper insertion of the needle into the vein. It is important that the needle used be sharp, that the bevel be neither too long nor too short, and that the tip of the needle be pointed rather than round. As a rule, a large (15 gauge) needle is used for the donor, an 18 gauge needle for adult recipient, 20 or 22 gauge needles for infants and small children. The skillful introduction of the needle into the vein is practically painless. However, particularly when the veins are small and difficult to enter, the intracutaneous injection of a small amount of 2 per cent procaine mitigates the discomfort of the procedure.

It is important to select the largest vein possible, and then place the limb in a position that will keep the vein stretched so that it does not slip to one side when the needle is inserted. A tourniquet is applied at a tension high enough to obstruct the venous but not the arterial flow, and then it is well to wait until the vein becomes distended. Good results can be obtained with the aid of the cuff of a blood pressure monometer adjusted to slightly above the diastolic pressure.

If the vein is deep set and immobile as at the bend of the elbow the needle may be plunged directly into it. Should the needle pass entirely through the vein, withdraw it gradually until blood flows freely from the hub. A snap may be felt as the needle point is dislodged from the posterior wall of the vein. Then depress the hub and insert the needle further into the vein. For superficial veins which tend to move the most satisfactory technic is the following. With the bevel of the needle uppermost, and with a slight downward and forward pressure the needle is pushed through the skin on a line with but not into the vein. During this procedure, the skin is kept stretched by drawing down with the balls of the fingers or thumb. The needle is kept as flat as possible and advanced approximately $\frac{1}{2}$ to 1 cm, to form a ridge over the upper surface of the vein wall. Then the butt of the needle is raised and the needle advanced slightly, whereupon it will enter the vein. Finally the needle is flattened out again to avoid perforating the far side of the vessel, and it is pushed up the vein for a distance of approximately $\frac{1}{2}$ to 1 cm.

In adults and older children there is rarely difficulty in finding suitable veins for transfusion. If it is impossible to enter the vein directly

through the skin, the veins can be exposed by incising the skin. The incision is usually made over the antecubital space, or if the antecubital veins cannot be used, an incision can be made above and slightly anterior to the medial malleolus of the ankle, where a suitable vein will be found. This may be necessary when the veins are poorly developed or collapsed, particularly in infants or in patients in shock. Even in very young infants, however, the antecubital or ankle veins may be so well developed that no incision need be made. In infants moreover, suitable veins may be found in the scalp after the hair is shaved and at times, the jugular vein can be used.

When the vein is exposed by an incision through the skin an ordinary intravenous needle can be inserted into the vein. However in order to avoid perforating the vein higher up it is better to use a blunt cannula with pointed stylette (Lindemann type). Such cannulas can be made from spinal tap needles by cutting across the needle 1 to 2 mm from its tip and beveling around the circumference to make it taper to the stylette. Such a cannula can be inserted without cutting the vein and it is not necessary to tie off the vein after the transfusion is completed.

On account of the difficulty of venepuncture in young infants transfusions have been given through the superior longitudinal sinus. This method of transfusion should no longer be used however since it is far safer to cut down on a vein. Transfusion through the longitudinal sinus is a blind procedure and there is a certain amount of risk of producing a subdural or intracerebral hematoma.¹ A number of fatalities from this cause have been reported.²

The intraperitoneal route has also been suggested for transfusions in infants. Since absorption takes some time the method is less useful in hemorrhage or shock. Moreover the retention of blood for a long time in the peritoneal cavity introduces the danger of infection. In a report published in 1929 Cole and Montgomery³ claimed good results in a series of 117 intraperitoneal transfusions. However there were reactions (abdominal discomfort restlessness etc.) in 7 per cent of the cases. These writers warn that the method should never be used in the presence of intra abdominal disease. When the intraperitoneal route is used the needle is inserted midway between the symphysis pubis and the umbilicus care being taken that the bladder is empty. The author has never resorted to the intraperitoneal route since he has always been able to find suitable veins even in newborn infants.

Tocantins and O'Neill⁴ report the successful infusion of blood and other fluids into the circulation by way of the bone marrow. In 17 trials there was only one failure. The needle was usually inserted into the sternal marrow but in 2 children the fluids were introduced into the tibia and femur. The authors recommend this technic where it is impossible to use the intravenous route as in widespread mutilations and burns. Experiments in rabbits on the occurrence of pulmonary fat

¹ J. G. Oliver *La Transfusion de Sangre en Pediatria* 178 pp. Buenos Aires (1937).

² Cf. Levine and Katzin *Jour Amer Med Assoc* 110: 1243 (1938).

³ *Amer Jour Dis Child* 37: 497 (1929).

⁴ *Proc Soc Exp Biol and Med* 45: 782 (1940). Tocantins and O'Neill *Surg Gyn and Obstet* 73: 287 (1941), Tocantins, O'Neill and Jones *Jour Amer Med Assoc* 117: 1229 (1941).

emboli after infusions into the bone marrow have been described by Wile and Schamberg^{4a}

In desperate cases, as in moribund patients, direct injection into the heart has been tried⁵

As to the question whether citrated or unmodified blood should be transfused, the most satisfactory and flexible system is to use both methods of transfusion, depending on the indication. In cases of acute hemorrhage, transfusions of unmodified blood by the syringe-valve method is the method of choice (cf page 105), as here speed is important. Besides it may be necessary to transfuse large quantities of blood, and the citrate method would then entail the injection of considerable quantities of the salt. On the other hand, in elderly patients and in patients with organic heart disease, and in other conditions where the rapid injection of blood might be dangerous, the transfusion of unmodified blood is contraindicated. In general, the citrate method will be found more satisfactory from the standpoint of patient, donor and operator, because of its greater simplicity and safety.

An attempt was made recently by Levine and Katzin⁶ to determine, with the aid of questionnaires, what methods of transfusion were being used in the hospitals of the United States. It was found that of 350 hospitals who replied to the questionnaires, 136 used one method exclusively, 172 used two methods, 34 used 3 different methods, and 4 hospitals used 4 different transfusion procedures. The following table gives the frequency of the four methods of blood transfusion used alone or in combination.

Citrate	306
Syringe-valve	189
Lindemann	62
Paraffin lined containers	46

It is seen that the citrate method is the most popular in American hospitals, evidently because of the ease of performance.

TRANSFUSION OF CITRATED BLOOD

The principle of this method of blood transfusion is to collect the blood of the donor in a vessel containing a citrate solution to prevent coagulation, and then to inject the blood into the recipient.

The Citrate Solution. As Neuhof and Hirschfeld⁷ state, as much as 6 to

^{4a} *Jour Invest Dermatol* 5: 173 (1942)

⁶ Tzanck, *Paris Med* 40/41: 249 (1922). With regard to other routes for blood transfusion, see: Moore and Dennis, *Northwest Med* 27: 140 (1928); Shaw, *Jour Amer Med Assoc* 90: 446 (1928)

⁷ *Jour Amer Med Assoc* 110: 1243 (1938)

⁵ *New York Med Jour.* 113: 95 (1921).

8 grams of sodium citrate may be injected intravenously without producing toxic effects provided that the injection is given slowly (over a period of 10 to 15 minutes) In blood transfusions by the drip technic even larger quantities are well tolerated Salant and Wise⁸ found that while sodium citrate when mixed with blood in vitro is stable, it is rapidly destroyed in the body In animal experiments it was found that the salt disappeared from the circulation very quickly, nearly 90 per cent being disposed of within ten minutes Part of the citrate is oxidized, the remainder (about 30 to 40 per cent) is excreted through the kidneys When toxic doses were injected symptoms appeared promptly, but if the dose was not lethal recovery was complete and without sequelae

Keynes⁹ points out that solutions of sodium citrate, being slightly alkaline in reaction, may attack the glass of the ampoule, and this in time gives rise to a whitish flaky deposit Therefore, it is best to keep the citrate in solid form and dissolve the salt in distilled water before use, but Riddell¹⁰ states that the solution in ampoules can be used with safety for three months after manufacture According to DeGowin¹¹ the use of isotonic citrate solutions is important for blood storage (cf page 135) DeGowin states that when the U S P XI preparation containing two molecules of water of crystallization ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) is used a 3.2 per cent solution is isotonic, while the salt with $5\frac{1}{2}$ molecules of water of crystallization is isotonic in a 3.8 per cent solution

In view of the harmlessness of sodium citrate in the doses required for transfusion, it is preferable to use the salt in some excess so that there may be no chance of coagulation of the donor's blood The author uses one part of a 3.8 per cent solution of the U S P salt¹² for every 10 parts of blood, so that in a 500 c c transfusion the total volume of the mixture of citrate solution and blood would be 550 c c The solution is prepared by dissolving 3.8 gms of the salt in 100 c c of distilled water, filtering and then sterilizing by autoclaving at 20 lbs pressure for 20 minutes

Technic ¹³ The apparatus recommended by Lewisohn for citrate transfusion is shown in figure 24

The first step consists in obtaining the blood from the donor A tourniquet is applied to the donor's arm and a large intravenous needle (15 gauge) is inserted into the most prominent vein (usually the median cephalic) at the bend of the elbow A cannula of large diameter is neces

⁸ *Jour Biol Chem* 28: 27 (1917)

⁹ Keynes *Blood Transfusion* Oxford Med Pub London (1922)

¹⁰ Riddell *Blood Transfusion* Oxford Univ Press London (1939)

¹¹ Personal communication

¹² According to DeGowin (cf footnote 11) this solution would be slightly hypertonic

¹³ See Lewisohn *Boston Med and Surg Jour* 190: 733 (1924)

sary in order that the blood may flow freely, otherwise clotting may occur before the blood is properly mixed with the citrate solution. If a large vein is available, it does not matter whether the needle points towards the hand or the shoulder. If the vein is small, it is preferable to point the needle towards the hand. The blood is allowed to flow into a large receptacle containing the necessary quantity of sodium citrate solution. The blood and citrate are mixed by gentle shaking of the re-

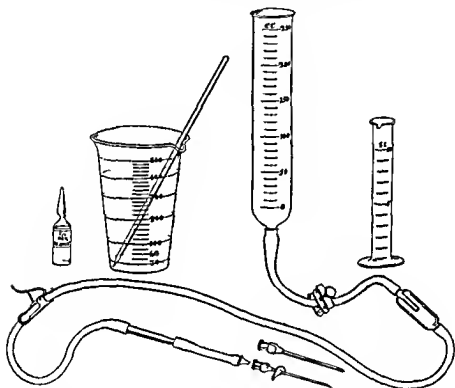


FIG. 24 APPARATUS FOR LEWISOHN'S CITRATE METHOD OF TRANSFUSION

ceptacle and stirring with a glass rod. Rough handling should be avoided to prevent damage to the blood corpuscles. When the full amount of blood has been drawn (usually about 500 c c), the tourniquet is released, the needle is withdrawn from the vein, a gauze pad applied, and the forearm is placed in complete flexion for a period of about 5 minutes.

The donor's blood can be collected more neatly and aseptically by making it run through a short piece of tubing connected to the needle with the aid of an adapter (cf. figure 25) instead of having the blood spurt directly from the needle into the cylinder. Before the needle is inserted into the donor's vein a hole large enough to admit the tubing

is punched through the paper covering the sterilized cylinder. As an additional precaution against coagulation when injecting the citrate solution into the cylinder prior to the blood collection, the last few c c can be injected through the tubing with the needle attached. Naturally, stirring is precluded by this technic but adequate mixing of blood and citrate will occur if the bottle is gently rotated in order to swirl the blood about.

When the procedure is properly performed the donor's blood flows in a steady



FIG 25 DRAWING BLOOD FROM DONOR (CITRATE METHOD)

stream and the collection of 500 c c takes only about 3 to 7 minutes. If the flow slows down or stops the tourniquet should be inspected and readjusted if it is too tight or too loose or the needle may have to be moved if its bevel is resting against the wall of the vein or the rubber tubing may have to be straightened if kinked. To maintain a steady flow the donor may be instructed to open and close his fist slowly but firmly and if necessary the forearm can be massaged with the flat of the hand from the wrist upwards towards the needle.

For the injection of the citrated blood, the set used for administering salvarsan by the gravity method is satisfactory (cf fig 26). The air in the tubing is first displaced by pouring some normal saline solu

tion into the apparatus. A tourniquet is applied to the arm, and the needle inserted into a prominent vein at the bend of the elbow, pointing in the direction of the shoulder. As soon as the needle has entered the patient's vein, as will be evidenced by the free flow of blood from the

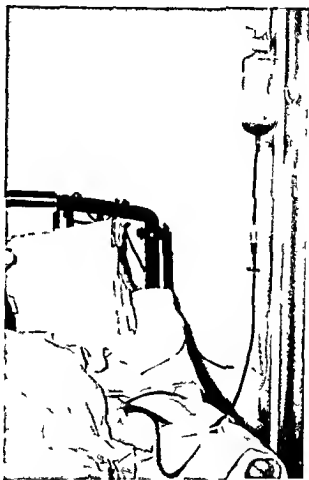


FIG. 26 TRANSFUSION OF CITRATED BLOOD BY GRAVITY METHOD

needle, the tourniquet is removed. The formation of a hematoma at the site of insertion of the needle usually indicates that the point of the needle is not in the lumen of the vessel; in that event the needle should be reinserted. The adapter of the salvarsan apparatus is then connected to the needle and the saline is allowed to flow in by gravity.

When no doubt remains that the intravenous infusion is flowing satisfactorily, the saline remaining in the reservoir is poured off and the

citratd blood is poured into the apparatus. If the blood has been properly collected it should contain no clots, and filtering the blood will then be neither necessary nor desirable. The final step is to adjust the regulator so that the blood flows at the desired rate, usually 10 c c per minute or about 3 to 4 drops a second.

Syringes or a syringe-valve apparatus can be used with advantage for



FIG 27 TRANSFUSION OF CITRATED BLOOD BY SYRINGE VALVE METHOD

the injection of citrated blood, instead of the gravity method (cf fig 27). In infants, the author prefers the syringe-valve method of injecting citrated blood, because the small caliber of the needle employed makes infusion by gravity rather tedious. Incidentally, in children requiring large amounts of blood but not urgently, it is convenient to store the citrated blood in the ice-box and give portions on 2 or 3 successive days.

Perhaps the greatest advantage of the citrate transfusion is its simplicity. Any physician can perform a blood transfusion by this method unassisted. Another practical advantage is that no special or elaborate apparatus is required. The ordinary intravenous equipment available in every small hospital will serve the purpose. Since there is no danger of clotting, the administration of blood can be regulated at any desired speed, so that the possible danger of overloading the heart from too

rapid administration of blood can be avoided. This makes it possible to transfuse larger than the usual amounts of blood at one time in cases with severe anemia. An additional advantage is elimination of personal contact between donor and recipient, thus preventing disturbances and excitement, particularly when family donors are used.

It has been claimed that a larger percentage of reactions (chills and fever) follow the use of citrated than of unmodified blood (cf. page 128), but in the author's experience¹⁴ there has been no difference in the incidence of reactions by the two methods. Moreover, as Lewisohn and Rosenthal¹⁵ have shown, such reactions can be prevented almost entirely by proper cleansing of the apparatus and the use of solutions prepared with pyrogen free distilled water¹⁶ (cf. page 130).

Continuous Drip Transfusion In patients (children or adults) who are actively bleeding, or where active blood destruction is going on, Marriott and Kekwick¹⁷ found continuous drip transfusion an ideal method of treatment and of preparing the patient for operation. The blood is drawn from several donors of the proper group into citrate solution in the usual manner. For adults, the rate of flow during the injection is regulated at about 40 drops per minute, so that approximately one liter of blood is transfused every seven hours. In children the rate of infusion should be regulated in proportion to the weight of the patient. Because of the slow rate of flow it will be found that the red blood cells have a tendency to sediment and plug the gravity apparatus, but Marriott and Kekwick were able to obviate this difficulty by bubbling oxygen through the blood at a very slow rate. These investigators advise that the transfusion be continued until the hemoglobin rises to an approximately normal level, after which a contemplated operation may be made. If required, as much as 5000 c.c. of blood can be transfused by this method into an adult within 36 to 48 hours. For further details concerning the technic see the original paper.

Such excellent results have been obtained by Marriott and Kekwick by the drip method that it may be in place here to cite one case from their series as an example.

A male aged 57 was suffering from severe hematemesis and melena of uncertain causation but thought to be due to gastric ulcer. When drip transfusion was decided upon he had been in the hospital for five days and was rapidly getting worse and continuing to lose blood. His condition appeared desperate and his hemoglobin was reduced to 20 per cent. Drip transfusion was carried out at a very slow rate and 47 hours were taken to administer 4100 c.c. of blood and 800 c.c. of

¹⁴Wiener, Oremland, Hyman and Samwick. *Amer Jour Clin Path* 11: 102 (1941).

¹⁵Lewisohn and Rosenthal. *Jour Amer Med Assoc* 100: 466 (1933).

¹⁶Seibert. *Amer Jour Physiol* 67: 90 (1923).

¹⁷*Lancet* 1: 977 (1935), *Proc Roy Soc Med* 29: 337 (1936).

citrate solution. The patient slept during most of the time without sedatives. At the conclusion his hemoglobin had risen to 103 per cent and his complexion was positively ruddy. His blood pressure was taken at intervals and slowly rose from 110/60 at the commencement of the transfusion to 150/75 at the end. In spite of this rise he had no more bleeding and made a smooth recovery.

Exsanguination Transfusion As has already been pointed out, in some conditions it is desirable to combine transfusion with the removal of some of the patient's own blood (cf. page 90).

The transfusion is started in the usual way, and after 200 or 300 c c of blood have been administered, a 15 gauge needle is inserted into a large vein in the opposite arm and 400 to 500 c c of the patient's blood are withdrawn. The transfusion can then be quickly completed. This procedure is preferable to withdrawing the blood before starting the transfusion, as very sick patients may react badly to the loss of blood and the amount of donor's blood lost by performing the phlebotomy after the transfusion has been started is negligible.

TRANSFUSION OF UNMODIFIED BLOOD

When transfusing unmodified blood it is necessary to transfer the blood from donor to patient before coagulation begins. Inasmuch as contact with foreign substances, particularly with rough surfaces, and with air favors coagulation, most modern methods of blood transfusion employ air tight apparatus with perfectly smooth surfaces, and the blood is passed through the apparatus quickly, remaining outside the body only for as short a time as possible.

More than a hundred different apparatus have been devised for transfusing unmodified blood but only a few representative types need be discussed here. In general, every transfusion apparatus can be placed into one of four categories: namely, variations of (1) the multiple syringe method, (2) methods employing paraffin lined containers, (3) the syringe valve methods, and (4) the tube methods. The simplest and most popular apparatus make use of either the syringe valve or tube principle. However, the other two more cumbersome procedures are still being used in a number of hospitals.

Multiple Syringe Method of Blood Transfusion This method of transfusion was perfected by Lindeman,¹⁸ and is really a revival of the Ziemssen method of 1892 (cf. page 56).

For this procedure six to twelve 50 c c syringes (depending on the quantity of blood to be transfused), intravenous needles of suitable gauges and practiced assistance is required. One operator draws the

¹⁸ *Amer Jour Dis Child* 6: 28 (1913) *Jour Amer Med Assoc* 62: 993 (1914), 72: 1661 (1919).

blood from the donor, a second operator injects it into the patient. A tourniquet is placed on the donor's arm, tight enough to obstruct the venous return but not the arterial flow (to be kept there during the entire procedure) and a needle inserted into one of his veins. Simultaneously, a tourniquet is applied to the patient's arm to distend the vein, a needle is inserted and the tourniquet released. The obturator is removed from the donor's cannula, an empty syringe attached and the blood withdrawn as quickly as possible. When the syringe is filled, one operator replaces it by an empty one, putting down the filled syringe. The other operator removes the obturator from the recipient's needle, picks up the filled syringe and attaches it, then speedily but smoothly injects the blood into the patient's vein. By the time the syringe is empty, a filled syringe is ready to replace it, and the process is repeated until the desired amount of blood has been transfused. It has been suggested that if there are not sufficient syringes for the amount of blood required, a third assistant rinse the used syringes with normal saline solution. If the same syringes are used repeatedly attention must be paid to the possibility of transmitting infection from patient to donor.

The main advantage of the above method is that no special apparatus is required. However, because of the ever present danger of coagulation, considerable practice is required to master the technic. To facilitate the procedure Hedenius¹⁹ has suggested that prior to the withdrawal of blood the blood donor be injected intravenously with sufficient heparin (0.25 to 1 mg per kg body weight) to prolong his coagulation time to 20 minutes. According to Hedenius the amount of heparin required is not large enough to harm the donor and does not affect the patient's coagulation time. In stressing the advantages of his method Hedenius states that only the simplest equipment need be used, since all the physician requires is a heparin ampoule and a syringe. However, further proof of the harmlessness of heparin injections would seem desirable.

Transfusion by Means of Paraffin Coated Containers. In this indirect method of blood transfusion, coagulation of the donor's blood is delayed by collecting it in a receptacle evenly coated with paraffin.

*Kimpton Brown Tube.*²⁰ The Kimpton Brown container is a large glass cylinder, possessing a perpendicular side tube a little below its upper end and an S shaped cannula at the bottom of the tube (cf. fig. 28). Double bulb rubber bellows are attached to the side tube. The original tube described by Kimpton and Brown had a capacity of only 100 c.c. but it can be made in any size, and the one mostly used has a capacity of 700 c.c.

Preparation and Care of Tube. Immediately after the tube has been

¹⁹ *Acta Med Scand* 89: 263 (1936); *Lancet* 2: 1186 (1937); *Cf. Sappington Jour Amer Med Assoc* 113: 22 (1939).

²⁰ Kimpton and Brown *Jour Amer Med Assoc* 61: 117 (1913).

used, it is washed out, first with cold and then with hot water. This washes the tube clean of blood, while the hot water helps remove most of the paraffin coating. The remainder of the paraffin may be removed by alcohol and ether. After all the ether has evaporated, the tube is ready for resterilization and reparafrinization. The tube may also be cleansed as described on page 131. A small piece of pure paraffin with a melting point of 50°C is then placed in the cylinder and a cork inserted. The tube is wrapped in a towel and sterilized in the auto-clave. The sterile tube is warmed over a Bunsen flame or an alcohol lamp, being carefully rotated in order to permit the melted paraffin to coat the entire inner surface of the tube, cork and side tube. The excess of paraffin is allowed to run out of the cannula. A small piece of sterile absorbent cotton is then inserted into the side tube to prevent contamination when the rubber bellows is used. To insure the formation of an even surface of the paraffin, the tube should be rapidly cooled by twirling it, for example, in front of an electric fan, until the thin film of paraffin has set.

Before the tube is used one must be certain that the lumen of the cannula is clear. If it is found plugged, the tip of the cannula should be reheated over a flame until the paraffin flows and the lumen is re-established. If one attempts to clear the lumen with a pin, the paraffin may crack, leaving part of the wall of the tube denuded.

Transfusion. One of the donor's veins at the bend of the elbow is exposed under local anesthesia, and an oblique incision is made into the vein. The flap thus formed is raised with a pair of forceps, and the tip of the cannula is inserted into the vein in the direction of the hand. When now a tourniquet is applied to the donor's arm, the blood will flow rapidly into the paraffin tube. If the tube is inserted so that its widest diameter engages the vein, no leakage of blood will occur. As soon as the required amount of blood has been obtained, the tourniquet is removed from the arm and the tube is withdrawn from the donor's vein. A ligature previously inserted about the vein distal to the point of incision is immediately tied by an assistant. Bleeding may also be prevented by pressure with gauze over the incision.

In the meantime an assistant has prepared the recipient's vein for

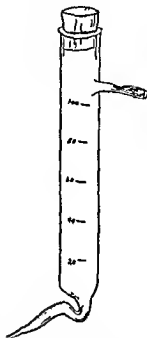


FIG. 28. KIMPTON AND BROWN'S CANNULA FOR TRANSFUSION
(100 cc. capacity)

insertion of the cannula. No time should be lost or the blood may clot in the paraffin tube. While the tube is being carried over to the recipient, the opening of the cannula should be closed with the gloved finger in order to prevent the escape of the blood. The cannula is inserted into the recipient's vein as described above, but with the tip pointing to the shoulder. The tube with the rubber bellows may now be operated as an aid in forcing the blood into the patient's circulation. Too much pressure on the bellows must be avoided. Towards the end of the transfusion the positive pressure must be released to avoid the introduction of



FIG. 29 KIMPTON AND BROWN'S CANNULA IN HORIZONTAL POSITION SHOWING THE TRAP WHICH PREVENTS THE ENTRANCE OF AIR INTO THE CANNULA

air into the recipient's circulation. After the cannula has been withdrawn from the recipient's vein, the vein is ligated and the skin of donor and recipient is sutured.

Disadvantages of the Paraffin Tube Method. The main disadvantages of the paraffin tube method of blood transfusion are due to the difficulties encountered in the proper preparation of the tube. If there is any defect in the paraffin coating, the blood will clot and the transfusion will be a failure. Another drawback of this method is that particular skill is required in its performance. Thus, the blood may clot if too much time is lost after its collection. As a rule only about ten minutes are required for the performance of the transfusion—seven minutes for the withdrawal and three minutes for the injection of the blood. Usually almost twice this time may be allowed to elapse with impunity. Other objections to this method of transfusion are that it is necessary to make an incision and to ligate the veins of both the donor and recipient and that the procedure cannot be performed without assistance.

Among the modifications of the Kimpton Brown tube the one devised by Percy¹ is probably most frequently employed in those hospitals which still adhere to the paraffin tube method. Percy has modified the apparatus so that not only a rubber bellows but also a suction tip is attached to the tube. During the withdrawal of the blood if the flow into the tube is retarded because of the back pressure of the blood an assistant applies gentle suction. As an additional precaution against coagulation a small amount of paraffin oil is drawn into the tube before it is inserted into the donor's vein. This oil layers the blood and prevents contact with the air.

¹ *Surg., Gyn and Obstet* 21: 360 (1915)

In Doghott's²² modification the veins are entered by means of needles just as in the syringe and citrate methods so that it is not necessary to isolate and incise the vein. The method of multiple paraffinized tubes is used here each tube having a capacity of 100 c c. In this way the likelihood of coagulation is diminished and if the procedure must be interrupted for any reason a large amount of blood is not wasted.²³

Transfusion by Means of Containers Made of Athrombit As has already been pointed out, perhaps the main disadvantage of the paraffin tube method of transfusion is the difficulty of properly paraffinizing the tube. Neubauer and Lampert²⁴ showed that if the apparatus is made of "athrombit" (an amber-like substance, which is a condensation product of phenol and formaldehyde), instead of glass, the containers need not be paraffinized. Apparatus made of athrombit can be sterilized without damage, the substance being resistant to heat and weak acids. Alkalis must be avoided, however.

Burkle de la Camp²⁵ has constructed an apparatus of athrombit that is similar in principle to Percy's paraffin coated tube. Transfusion apparatus made of athrombit are used in Germany.

Blood Transfusion by the Syringe-Valve Method This is the most popular method of transfusing unmodified blood at the present time. The choice of a particular apparatus is largely a matter of personal preference. To illustrate transfusion by this method, the procedure followed by Unger²⁶ will be described.

Unger's Method The essential part of Unger's apparatus is a syringe with a four way stop cock. The technic is reliable, easy to learn, and has become very popular.

One opening of the stop cock is connected to a syringe for blood, and another to a syringe containing saline. There are also attachments for tubings leading to the donor and recipient respectively. The stop cock has two positions. In the donor's position, the syringe for blood is connected to the vein of the donor, and the syringe for saline to that of the recipient. In the recipient's position, conditions are reversed (cf. fig. 30).

Technic The donor and recipient lie parallel to each other with their arms resting on a board supported between them. A tourniquet is applied to the recipient's arm, and a vein entered by means of a small intravenous needle. The tourniquet is then removed, and the needle

²² For a description of this method see Thorek, *Surgical Errors and Safeguards* p. 40 J. B. Lippincott Co. (1932).

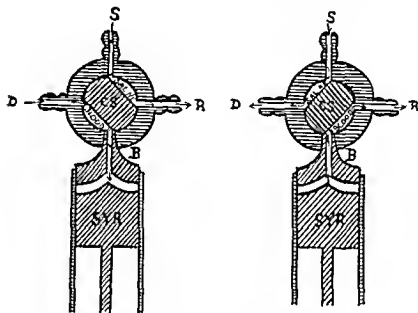
²³ Other modifications have been advocated by Kreuscher [*Jour Amer Med Assoc* 70: 223 (1918)] Brooks [*Calif and West Med* 24: 44 (1926)] and many others.

²⁴ *Munch med Woch* 77: 582 (1930).

²⁵ *Zentralbl Chir* 58: 854 (1931).

²⁶ *Jour Amer Med Assoc* 65: 1029 (1915).

connected to the adapter at the end of the recipient's tube. The stop-cock is set in the donor's position, and an assistant very slowly injects saline into the recipient's circulation (to prevent clotting). A tourniquet is applied to the donor's arm, and a prominent vein is chosen into which a large needle is inserted and immediately connected to the donor's tube. The donor's blood is then aspirated by the operator. As soon as the syringe is filled, the stop cock is turned and the blood is injected into



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FIG 30 UNGER'S TRANSFUSION APPARATUS

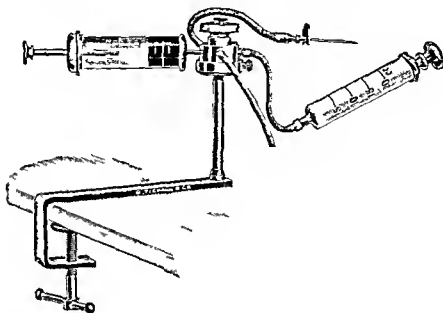
When apparatus is in donor's position (left) the blood passes from the donor's vein through D, out at B into Syr. At the same time saline is being forced from the saline syringe through S out at R into the recipient's tube. In the recipient's position (right) the blood is forced out of Syr through B out at R into recipient's circulation. At the same time the donor's tube is kept clear with saline.

CS central stopper (rotates through an arc of 90 degrees)

the recipient. The stop cock is then turned back to the donor's position and this procedure is repeated until the desired amount of blood is injected. A 10 to 20 c.c. Record syringe is usually employed so that the amount of blood can easily be calculated at any time.

During the transfusion, an assistant alternately flushes the circuit of donor and recipient by slowly injecting saline. A small amount of saline for instance 20 c.c., should be sufficient for a transfusion of 500 c.c. of blood. As soon as the syringe for blood begins to work with difficulty,

the stop cock is turned to the intermediate position to prevent the loss of any blood and the syringe is removed and replaced by a clean one. Coagulation of blood during the transfusion may be inhibited by spraying ether on the outside of the syringe. For this purpose a small can of ether in the top of which a very small pin point opening has been made may be used. The spray is facilitated by wrapping a hot towel about



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FIG. 31. UNGER'S APPARATUS

the can. In this way it may be possible to perform the entire transfusion without changing the syringe.

Other Methods of Syringe Transfusion. In practically all of the modifications of the method just described the special syringe for saline is dispensed with so that the operator can perform the transfusion unaided. This can also be accomplished with the Unger apparatus by clamping the tube leading to the saline syringe. In Scannell's²⁷ modification the syringe is connected to a three-way stop cock with connections leading to donor, recipient and a basin of saline. As soon as the syringe begins to work with difficulty it is flushed out with saline so that frequent changing of the syringe is avoided. Brines²⁸ has modified Unger's technic by using the saline syringe for blood so that a constant flow of undiluted blood is maintained from donor to recipient.

²⁷ Scannell *Blood Transfusion Simplified* Brockton, Mass. (1930).

²⁸ *Arch. Surg.* 12: 124-140 (1926).

Feinblatt²⁹ states that Unger's apparatus is open to the theoretical objection that the presence of a chamber where the stop cock rotates may possibly allow reflux of blood from recipient to donor. He has therefore devised an apparatus in which the essential part is a two way valve operating by means of two discs rotating on one another through an angle of 90°, thus eliminating the chamber.

A number of other transfusion apparatus (Miller Tzanck etc.) have been devised recently in which the essential part is a sleeve valve. In Miller's apparatus (cf

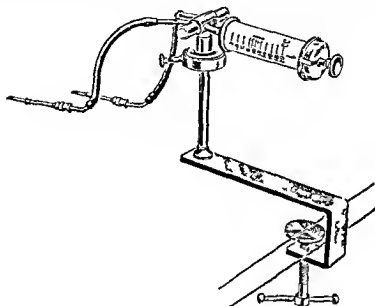


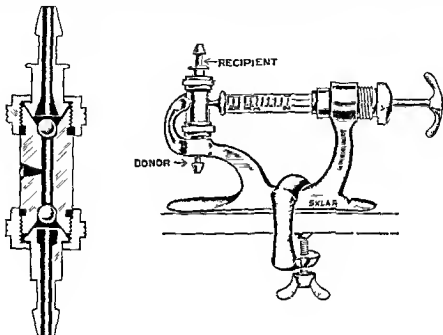
FIG 32 MILLER'S TRANSFUSION APPARATUS

figure 32) the valve is built like an all metal syringe mounted on a table clamp. The plunger has a perforation in its center connected at the proximal side with a metal nipple into which the syringe is inserted and on the distal side to an inlet or an outlet tube depending on the position. By moving the plunger with the attached syringe to and fro aspiration and injection is accomplished.

The syringe valve method of transfusing unmodified blood has the advantage that a single operator can carry out the procedure unaided and with expediency. On the other hand, care must be taken to keep the apparatus in good condition (to prevent leakage). Moreover, a certain amount of practice is needed in handling the apparatus, particularly to guard against turning the valve in the wrong direction and reversing the direction of the flow.

²⁹ Feinblatt *Transfusion of Blood*, p. 106 New York (1926)

Soresi³⁰ and Koster³¹ have each invented an apparatus by which the blood is aspirated and injected in 2 cc quantities. The essential part of Soresi's apparatus is the blood flow controller (cf fig 33) which automatically controls the flow of blood from donor to recipient. The blood flow controller consists of a body two small metal balls two plugs and two lock nuts which may readily be taken apart and reassembled. When the apparatus is in position the two balls fall to their seats by gravity and close the channel cut through the body of the controller. When the syringe aspirates the lower ball is drawn up thus allowing the donor's blood to fill the syringe and the upper ball is tightened against its seat effectively closing the



Reproduced by courtesy of J. Sklar Manufacturing Co.

FIG 33 SORESI'S APPARATUS

channel to the recipient. After the syringe is filled the lower ball falls against its seat closing the channel leading to the donor. When the blood is expelled from the syringe the upper ball is forced upward opening the channel to the recipient while the lower ball is fastened in its seat. This alternate lifting and falling of the upper and lower balls automatically controls the flow of blood from donor to recipient so that no valves need be operated. Soresi claims that this method also has the advantage that coagulation is prevented because of the small size of the syringe so that the blood is in the apparatus only a very short time. For this reason the same syringe can be used for the entire transfusion. In the Soresi and Koster apparatus the valves operate automatically so that the apparatus is simple to manipulate even for the beginner. The theoretical objection has been raised that the valves might be jammed by a plug of fibrin without the operator's knowledge and in that way the flow of blood could be reversed.

³⁰ Jour Amer Med Assoc 84: 591 (1925)

³¹ Med Jour and Rec 122: 286 (1925)

Tube Method Recently apparatus have come into use by which the blood is transferred from donor to patient through a tube, without the aid of syringes. These apparatus have considerably simplified the transfusion of unmodified blood and would deserve to be generally adopted. In Germany, an apparatus invented by Beck²² is widely used. The essential part of Beck's apparatus is a strong rubber tube connected to



FIG. 34 THE BAKER TRANSFUSER

needles in the veins of donor and recipient, through which the blood is milked with the aid of rollers. The elasticity of the rubber tube causes the donor's blood to be aspirated, and the rollers force the blood into the patient's circulation. Similar apparatus have been introduced into the United States by DeBakey²³ and by Baker²⁴ (cf fig 34).

²² *Ergeb inn Med u Kinderheilk* 30 150 (1926)

²³ DeBakey *New Orleans Med and Surg Jour* 87 386 (1934)

²⁴ Cashman and Baker *Amer Jour Obst and Gyn* 31 240 (1936)

An ingenious apparatus which works by a somewhat different mechanism has recently been invented by Pennell³⁵ In Pennell's method, the blood is forced through a continuous tube with the aid of a spiral metal screw instead of rollers (cf figure 35) To reduce the friction between the metal screw and the tubing the apparatus is filled with sterile glycerine before use The tubing is calibrated to deliver 2 c c of blood for each turn of the handle and an automatic counter measures the amount of blood transfused A ratchet permits the handle to be

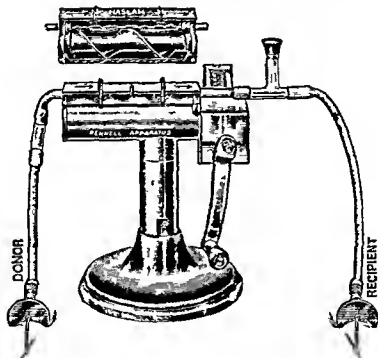


FIG 35 PENNELL TRANSFUSION APPARATUS

turned in only one direction in order to prevent reversal of the flow of blood

Pennell's method has the following advantages over the syringe valve methods of blood transfusion The apparatus is so simple to operate that the method requires not much more skill than a venepuncture The apparatus is easily cleansed has no breakable glass parts and the only thing that need be replaced is the inexpensive rubber tubing The theoretical objection has been raised that the friction on the tubing may rub off particles of rubber from the inner lining which might be injected into the patient This is avoided however, by the use of specially treated rubber tubing supplied with the apparatus

³⁵ Pennell *Amer Jour Surg* 45 354 (1939)

The tube method of transfusion has the disadvantage that it is not possible to build up as much pressure as with the syringe valve technic. Therefore a larger needle must be used for the patient (16 or 17 gauge) and if any resistance develops on the patient's side or the crank is turned too rapidly the apparatus will deliver less than the calibrated amount. In addition one must keep one's eye on the tubing connected to the donor's vein since if the flow slows down or stops the only indication may be the collapse of this tubing.

The tube method, like the syringe valve method, can be used for transfusing citrated blood as well as unmodified blood. Where the transfusion has been started by the gravity method and it becomes necessary to speed up the infusion Pennell has found that this is readily done by slipping the intravenous tubing into his apparatus and turning the crank at the desired speed.

DOSAGE AND RATE OF TRANSFUSION

It can be shown that the injection of 15 c.c. of normal blood for every kg. body weight will increase the red cell count by approximately one million cells per cu. mm. The usual transfusion in adults consists of 500 c.c. of blood, and this for an individual weighing 70 kg. (approximately 150 lbs.) would produce a rise in cell count of 500,000 and in hemoglobin percentage of about 8 to 10. Depending on the indication the dosage may vary from 200 c.c. to 1000 c.c. or more since as was pointed out in chapter VI, in some conditions beneficial results are only obtained by injections of large amounts of blood, while in other conditions repeated small transfusions are preferable.

In children the dosage is usually higher in proportion to body weight than in adults, 15 to 20 c.c. of blood being given per kg. body weight. An infant weighing 11 lbs. (or 5 kg.), for example, would receive a transfusion of 75 to 100 c.c. of blood.

In transfusions of unmodified blood the blood injection must be carried out with sufficient smoothness and rapidity so that clotting does not occur, at the same time it should not be injected rapidly enough to embarrass the heart. In adults, the blood is usually injected at a rate of 50 to 100 c.c. per minute. Immediately after a hemorrhage blood may be injected at even higher rates, without danger to the patient. Where the blood must be given more slowly, the citrate method is used and then a rate of 10 c.c. per minute will be found satisfactory in most cases.

CHAPTER VIII

REACTIONS AND COMPLICATIONS FOLLOWING BLOOD TRANSFUSION

THE REACTIONS to blood transfusion can be classified as (1) hemo-
lytic reactions (2) simple febrile reactions, and (3) urticarial and
other allergic reactions of which the first type is the most serious. In
addition certain complications can result from transfusion, for ex-
ample, overloading of the circulation, thrombosis of the punctured
vein and thrombophlebitis and embolism. Overloading of the circula-
tion due to the transfusion of too much blood or the too rapid injection
of blood particularly in individuals with cardiac disease, is probably
one of the most dangerous complications of transfusion, and has been
responsible for a number of fatalities.

In table 11 are presented some of the published reports on deaths
resulting from blood transfusion. It will be seen that the percentage

TABLE 11
FATAL REACTIONS FOLLOWING BLOOD TRANSFUSIONS

Investigator	Year of Report	Number of Transfusions Performed	Number of Deaths
<i>I Fresh Blood</i>			
Bernheim	1917	800	10
Pemberton	1919	1 036	3
Copher	1923	245	2
Kordenat & Smuthies	1925	764	0
Witts	1929	3 430	5
Brines	1930	4 000	2
Polayes & Morrison	1932	1 500	9
DeGowin	1938	3 500	7
Wiener et al	1941	4 000	1
Total		19 275	39
<i>II Stored Blood</i>			
Diggs & Keith	1939	1 415	5
	1940	2 140	1
DeGowin & Hardin	1940	1 604	2
Hoxworth & Skinner	1941	3 077	1
Total		8 236	9

of fatalities varies greatly with different operators some reporting few or no deaths others as many as 1 per cent. Moreover when the fatality rates are averaged the paradoxical result is obtained that the figures for transfusions of stored blood (0.1 per cent) are lower than those for the transfusions of fresh blood (0.2 per cent). The probable reason for these discrepancies and the apparent paradox is as follows. It will be seen that half the fatalities occurred during the period 1917-1930 though this includes less than one third of the transfusions listed. It is easy to understand why the fatality rate should be higher during the earlier years considering that the technics of blood grouping and blood transfusion were still in the process of development. For the most recent period (1938-1941) the average fatality rate reported is only 0.1 per cent. As for the great difference in the percentage of deaths reported by various workers this is readily explained by the difference in technics. In transfusions of unmodified blood by the multiple syringe or syringe valve method the blood must be injected within a few minutes while transfusions of citrated blood can be carried out as slowly as desired. Accordingly the danger of sudden death from overloading of the circulation is considerably greater when unmodified blood is given and it would seem probable that the main cause for the recent decrease in the number of fatalities following transfusions is the more general adoption of the citrate method and the resulting diminution in the danger of congestive heart failure.

As regards the other prominent cause of transfusion fatalities namely hemolytic reactions the statistics are not entirely reliable. While acute cardiac failure immediately following transfusion can hardly be overlooked when fatalities occur after several days from uremia caused by a hemolytic reaction the connection between the death and the transfusion may not be recognized. This explains why until recently deaths from uremia following transfusions particularly in pregnancy cases were erroneously believed to be due to complications of pregnancy e.g. toxemia rather than to the transfusion.

HEMOLYTIC REACTIONS

Etiology. Hemolytic transfusion reactions fall into two categories namely (1) those due to specific incompatibility between the bloods of donor and recipient and (2) those caused by non specific agents as when blood that has been stored too long is used for transfusion. Incompatibility may result from the unintentional selection of a donor of a different blood group from the patient or less frequently it may be caused by intragroup differences.

It is fortunate that the transfusion of blood of an incompatible

group, due to errors in grouping which regrettably still occur,¹ does not always cause serious reactions, indeed not infrequently the symptoms are so mild that they pass unnoticed or are attributed to other causes.² Hence, the fact that the donor's blood has been used previously without apparent ill effect does not prove that the original grouping tests were correct.

Some of the reports of supposed changes in blood groups have been based on clinical observations of unexpected hemolytic reactions following transfusions. The usual story is that a donor who had previously given blood to the patient without ill effect was used again after an interval of time and a severe or fatal hemolytic reaction resulted. Re-examination of the bloods of donor and patient may reveal for example that the donor belongs to group A, the patient to group O, not both to the same group as believed. The conclusion that used to be drawn was that there had been a change in blood group of patient or donor, while the correct explanation is that an error in grouping had been made. In fact, hemolysis of the donor's blood undoubtedly occurred even after the first transfusion but was overlooked because of the mildness of the symptoms. The severe symptoms following the second transfusion can be explained by the increased titer of the isoantibodies as a result of isoimmunization (cf. page 64).

The reason why an initial transfusion of blood of an incompatible group is not always followed by severe symptoms most likely is that in some individuals the isoantibodies are only of low titer. Moreover in certain diseases such as leukemia the isoantibody titer is depressed and in one leukemic patient of group O as much as 1500 cc. of group B blood was unintentionally transfused without any noticeable symptoms though the donor's cells were destroyed and eliminated from the body within a few days.³ Another fortunate feature is that just those properties of the blood which are responsible for the common mistakes also diminish the danger. Not infrequently donors of subgroup A₂B have erroneously been assigned to group B because the A receptor was not detected with the weak group B testing serum that was used. However, since the A receptor is weak, hemolysis *in vivo* will tend to be slow and cause only mild symptoms unless the recipient has exceptionally potent A isoantibodies. In this connection for a patient of subgroup A₂B whose serum contains an atypical agglutinin α_1 , group B blood should be given in preference to blood of group AB unless a donor of subgroup A₂B is available.

Special attention is called to errors in the grouping of professional donors by donor agencies. Recently the writer examined a blood donor whom he found to belong to group AB. Thereupon the donor, a medical student, stated that he had previously been typed as group O and had given three transfusions under this classification but as far as he knew there had not been any harmful effects from these transfusions. Investigation revealed that in one case the incompatibility between the patient's serum and donor's cells had been detected in the cross matching.

¹ In a recent report Seggel [*Med Welt* 2: 51 (1938)] remarks that in a series of 2105 blood transfusions carried out at a large hospital as many as 8 mistakes in blood grouping were found to have been made. Cf. Moureau, *Ann de Med Leg* 15: 951 (1933); Tzanck, Moline and Paris, *Bull et mem Soc d'hop de Paris* 49: 1297 (1933); Wiener et al., *Amer Jour Clin Path* 11: 102 (1941) etc.

² Wiener et al., *loc cit*.

³ Wiener and Schaefer, *Med Clin of N Amer* 24: 705 (1940).

test and only the donor's plasma had been used for the transfusion. In the other 2 cases where the donor's whole blood had been transfused one patient died within 4 hours the other within 4 days with symptoms and signs of transfusion hemolysis. These two transfusions had been given postoperatively and the deaths had been ascribed to the underlying diseases and operative procedures while the transfusions were not suspected to have any relation to the fatal outcome.

While the vast majority of the hemolytic reactions reported have been due to the presence in the donor's blood cells of incompatible agglutinogens there have also been a few reports⁴ of hemolytic reactions due to the transfusion of blood containing incompatible isoagglutinins, such as group O blood to patients of group A, B or AB. This question concerning the use of universal donors is discussed in greater detail on pages 60 and 117. Interest in this problem has been revived recently in connection with plasma and serum transfusions and measures have been devised to prevent possible damage to the patient's blood cells by isoantibodies in such blood substitutes (cf. page 150). It is generally accepted that when dangerous universal donors, i.e. donors with potent isoantibodies in their plasma are used the possibility of hemolysis exists. However recent work indicates that the danger from the indiscriminate use of universal donors is not as great as formerly believed.^{5a} According to Riddell,⁶ in France universal donors have been used almost exclusively, yet no fatality is said to have occurred in the experience of the Transfusion Sanguine D'Urgence in Paris which used to supply universal donors for as many as 6,000 transfusions a year.

As has already been pointed out (cf. page 65) hemolytic reactions can result from transfusions of blood of the same group as the patient due to intragroup differences. While sporadic reports have appeared in the literature of hemolytic reactions following transfusions of the homologous group this subject has been clarified only during the past few years. In the first systematic study of such reactions Wiener and Peters⁷ remarked that the isoantibodies responsible result from i-o

⁴ Muller and Balgaires *Compt rend Soc Biol Paris* 121: 1447 (1936). DeGowin *Jour Amer Med Assoc* 108: 296 (1937). Klendshoj and McNeil *Jour Amer Med Assoc* 118: 528 (1942). Wiener and Moloney *Amer Jour Clin Path* in press.

⁵ Related to this problem also are reports of hemolytic reactions following treatment of type XIV pneumonia with therapeutic horse serum due to the presence in human cells of an antigen related to the type XIV pneumococcus polysaccharide [Finland and Curnen *Science* 87: 416 (1938)].

⁶ Rosenthal and Vogel in Mudd and Thalhimer's *Blood Substitutes and Blood Transfusion* Chapter xxx. C. C. Thomas (1942). Cf. Aubert, Boorman and Dodd *Brit Med Jour* 1: 659 (1942).

⁷ Riddell *Blood Transfusion* p. 48. Oxford Univ. Press (1939).

⁸ *Ann Int Med* 13: 2306 (1940).

immunization which occurs in two classes of patients, namely, (1) patients receiving repeated transfusions and (2) pregnancy cases where the fetus *in utero* supplies the foreign antigen.⁸ The factor most frequently responsible for intragroup isoimmunization and consequent transfusion hemolysis has proved to be the agglutinin Rh of Landsteiner and Wiener. However, agglutinin Rh is a poor antigen for man in comparison to agglutinogens A and B, so that isoimmunization against Rh occurs relatively infrequently even in cases where this is theoretically possible, namely, when an Rh negative patient is given repeated transfusions of Rh positive blood or when an Rh negative woman has an Rh positive child. The infrequency of intragroup hemolytic reactions and the difficulties in demonstrating the incompatibility by *in vitro* tests are the probable reasons why this problem had not been solved previously. While intragroup hemolysis due to the Rh factor is an uncommon phenomenon, many more reactions of this sort have been reported than from the indiscriminate use of universal donors. Accordingly, a postpartum patient would be safer if given blood from an Rh negative universal donor than with blood of her own group selected without taking the Rh factor into account.

In the light of the recent observations on intragroup hemolytic reactions due to the Rh factor it is necessary to critically review the reports of hemolytic reactions following transfusions of universal donor blood. If the accepted explanation that such reactions are all caused by the action of incompatible isoantibodies on the patient's cells is correct, then in ordinary grouping tests after such transfusions as in the method used by Ashby to trace the fate of the transfused cells (cf. page 73), the donor's cells should be demonstrable since they are not agglutinable, but in none of the previous reports was this simple test mentioned. This leaves open the possibility that actually the donor's cells, not the patient's cells, were hemolyzed. The occurrence of a number of these reactions in patients receiving repeated transfusions or in postpartum cases suggests that isoimmunization to the Rh factor may have in part been responsible. Moreover, when group O blood is transfused to group AB patients of subgroup A₁B, hemolysis may possibly result from the (rare) presence of natural or immune anti O isoantibodies in the serum of the patient, as in a case seen by the author.⁹ For this reason, with patients of subgroup A₁B (with

⁸ Isoimmunization in pregnancy was mentioned as an explanation for a hemolytic transfusion reaction for the first time by Levine and Stetson [*Jour Amer Med Assoc* 113: 126 (1939)] and was independently considered by Wiener [*Jour Immunol* 41: 81 (1941)].

⁹ Wiener, Oremland, Hyman and Samuels. *Amer Jour Clin Path* 11: 102 (1941).

anti O agglutinins) where donors of the same group are not available, blood of subgroup A₁ or group B should be used in preference to group O blood

It has been assumed without proof that when hemolysis follows transfusions of group A blood to group A patients subgroup differences are responsible but in the cases observed by the author the Rh factor proved to be the cause. As a rule transfusions of A₁ blood to A₂ patients or vice-versa are entirely compatible the transfused cells surviving as long as blood of the homologous subgroup and this method has even been used by the author for tracing the fate of the transfused erythrocytes (cf page 76). There is evidence¹⁰ however that A₂ individuals can on rare occasions form immune anti A₁ isoantibodies either following transfusions of A₁ blood or as a result of pregnancy with an A₁ fetus and in such cases disregard of the subgroups might possibly result in hemolytic reactions. The irregular agglutinins α_1 and α_2 (or anti O) sometimes present in normal serum of subgroup A₂ (or A B) and A₁ (or A₁B) respectively would probably be less apt to cause serious reactions on account of their lower titer (cf page 45) though here also blood of the homologous subgroup may be preferable.

It has also been assumed without proof that hemolytic reactions following repeated transfusions are due to isoimmunization against M and N but the properties M and N probably play even a less important role than the subgroups of groups A and AB. To date anti M agglutinins have been found in only 5 normal human sera many hundreds of thousands tested (cf page 219) while immune anti M isoantibodies have been observed in only two post transfusion sera.¹¹ anti N agglutinins have never been found in any human sera. Therefore agglutinin M is a worse antigen for man than Rh while agglutinin N according to the available evidence is not antigenic in man. That possibly on rare occasions the property P may be responsible for intragroup hemolytic transfusion reactions is evidenced by the presence of anti P agglutinins in two post transfusion sera examined by the author. As is pointed out on page 65 on rare occasions other unclassified factors may also give rise to transfusion hemolysis.

As for the qualities of isoantibodies which affect the severity of hemolytic transfusion reactions, it appears that in general immune isoantibodies are of greater significance than natural isoantibodies. While there is a rough correlation between the titer of the isoantibody in *in vitro* tests and the severity of the reaction, serious hemolytic reactions have occurred in absence of any demonstrable isoantibodies. Presumably, the antibodies in such cases are bound to cells, perhaps reticuloendothelial cells. And, apparently other properties of the isoantibodies are of greater significance than their behavior *in vitro* with respect to temperature, for severe reactions have resulted when the isoantibodies in the patient's serum had properties of "cold" agglutinins as well as in cases with such antibodies behaving like warm agglutinins.

¹⁰ Wiener *Jour Immunol* 41 81 (1941)

¹¹ Wiener and Forer *Proc Soc Exp Biol and Med* 47 215 (1941) Wiener *Amer Jour Clin Path* 12 302 (1942)

Autoantibodies, in contrast to isoantibodies, seem to have little or no significance for the outcome of transfusions. The present author has observed many patients whose sera contained autoagglutinins active at room temperature *in vitro* and who were transfused without the occurrence of any reaction, there being the usual rise in hemoglobin following the transfusion. Antopol, Appelbaum and Goldman¹² describe two patients with acute hemolytic anemia (due to sulfanilamide therapy) who responded satisfactorily to transfusion therapy despite the presence of autoagglutinins in their sera. The author has observed a case of acute hemolytic anemia due to paroxysmal hemoglobinuria in a colored syphilitic woman in which the hemoglobin concentration of the blood was raised without difficulty from 25 per cent to its level before the attack of about 80 per cent despite the presence of autoantibodies in the patient's serum (positive Donath Landsteiner reaction). Hemolytic transfusion reactions occurring in patients with hemolytic icterus given repeated transfusions have been attributed to autoantibodies known to be present in such cases (cf page 86), but the possibility of isomunization also must be considered as an explanation. However, that autoantibodies of a special type may on rare occasions be responsible for hemolytic transfusion reactions cannot yet be excluded entirely (cf page 42). In all cases with strong autoagglutination, the author carries out a biological test using a small amount of the prospective donor's blood, and an attempt is made to keep the blood warm during the infusion, care being taken to avoid overheating.

Pseudoagglutination (or rouleaux formation) has not been found to play a significant rôle in post transfusion reactions.

Finally, it must be mentioned that hemoglobinuria, occasionally associated with oliguria or anuria with retention of non protein nitrogen, has followed transfusions of blood hemolyzed as a result of exposure to excessive heat or freezing or due to prolonged storage.

Symptoms¹³ The signs and symptoms of transfusion hemolysis vary considerably in different patients, in some being mild or inapparent, in others severe, and in rare cases causing almost immediate death.

A typical hemolytic transfusion reaction has the following course. During the injection of the blood, sometimes after the injection of as little as 10 c c. of blood, the patient complains of fullness in the head, precordial oppression, generalized tingling sensations and later of a sudden sharp pain in the lumbar region, the latter symptom being

¹² *Jour Amer Med Assoc* 113 488 (1939)

¹³ Cf Bordley *Arch Int Med* 47 288 (1931) Goldring and Graef *Arch Int Med* 58 825 (1936)

pathognomonic of the transfusion of incompatible blood. Inspection of the patient during the reaction reveals suffusion of the face, distention of the veins of the neck, restlessness and anxiety, dyspnea and coughing. Collapse may follow, with rapid feeble pulse and cold clammy skin, nausea and vomiting, at times resulting in death. Naturally, these symptoms may not be noticed in patients under the influence of an anesthetic. On the appearance of any untoward symptoms, the transfusion must be discontinued at once, and if collapse supervenes $\frac{1}{2}$ to 1 cc of epinephrine hydrochloride (1:1000) should be administered by hypodermic and repeated as required until the patient rallies. As a rule, within an hour after the onset of the reaction there is a severe chill, followed by a sharp rise in temperature, and this terminates what may be considered the first phase of the reaction. In some patients there is no immediate reaction to the transfusion but about one hour after the transfusion the reaction begins with a chill and fever as the outstanding symptoms. Not infrequently a hemorrhagic tendency develops immediately after the transfusion, and blood may ooze from the site of the transfusion, from the gums, uterus (in postpartum cases) or any fresh puncture made in the skin for the purpose of treatment.¹⁴ If a urine sample is obtained at the end of the first phase the urine will be found to be dark red in color, containing free hemoglobin but few or no red cells.

If the patient survives the immediate reaction, there follows a period of apparent improvement (known as the interval phase) but associated with oliguria or complete suppression of urine. Icterus may be noticeable on the day following the transfusion, and this usually disappears within a few days. There is apparently no simple relationship between the depth of the icterus and the amount of blood transfused. In the meantime, because of the anuria nitrogenous waste products accumulate in the blood stream, and after a variable interval the symptoms of uremia appear, usually quite suddenly. All the signs and symptoms of renal failure are now evident (renal phase), stupor, convulsions and coma set in, often associated with generalized edema and purpura. Recovery occurs in about half the cases, this being heralded by the abrupt onset of diuresis.

As has already been mentioned, in atypical cases the immediate reaction is lacking or overlooked, the first sign being a chill. In other cases, the chill and rise in temperature may be so mild as to be un-

¹⁴ It is of interest to note that after anaphylactic shock the blood has a tendency to coagulate slowly or not at all. See Arthur [Arch Intern Physiol 7: 471 (1909)], Biedl and Kraus [Wien klin Woch 22: 363 (1909)] and review by Eagle [Medicine 16: 125 (1937)].

noticed, and the existence of a transfusion reaction not be suspected until the patient goes into uremia. On the other hand, some patients recover completely from the immediate reaction and never develop any signs or symptoms of renal insufficiency, especially when the quantity of incompatible blood injected is small. In a few cases, the appearance of urticarial eruptions on the skin have been noted and on rare occasions petechiae have been observed immediately following transfusions of incompatible blood. The petechiae have been attributed to occlusion of small arterioles by agglutinated cells, and this is believed to be particularly apt to occur on repeat transfusion of blood of an incompatible group (as A to O) on account of isoimmunization. In such cases, moreover, almost instantaneous death may occur due to massive agglutination of the donor's cells with cerebral and cardiac embolism.

Diagnosis Because of the variability of symptoms of transfusion hemolysis in different patients, such cases not infrequently offer difficulties in diagnosis. The chill and fever of a hemolytic reaction may be considered a simple rigor and, in fact, the symptoms at times are so mild as to be entirely unnoticed. Uremia of transfusion hemolysis occurs so long after the transfusion that its connection with the transfusion may not be suspected, and the patient's death in such cases has often been ascribed erroneously to the underlying disease. On the other hand, in septic patients, chills occurring shortly after the transfusion are sometimes coincidental and caused by the infection rather than by the transfusion. The author has observed the occurrence of hemolytic anemia in a septic patient which was ascribed to blood transfusion, but was actually caused by sulfonamide therapy. Finally, it is difficult to decide whether immediate death following a transfusion due to incompatibility of the bloods or to acute cardiac failure.

When a reaction follows a blood transfusion, a sample of the patient's blood should be drawn at once, preferably during or shortly after the chill, in order to determine whether or not there has been hemolysis. The serum separated from the blood clot will usually contain free hemoglobin if the reaction was due to hemolysis. Oehlecker¹⁵ believes that in most of the cases reported in which no hemoglobin was found in the patient's serum the results were not valid because the patient's blood was not examined soon enough. In animal experiments in which Oehlecker transfused incompatible blood, he found that the concentration of hemoglobin in the serum reached its maximum within a minute, then rapidly fell for a few minutes until it had dropped to half the maximum value. The remainder of the hemoglobin was excreted much

¹⁵ Oehlecker, *Die Bluttransfusion* Vienna (1933)

more slowly, but within 4 or 5 hours as a rule, there was no longer any demonstrable hemoglobinemia. Following transfusions of old bank blood in man, the present author has observed that the patient's serum during the first two or three hours contains free hemoglobin; within a few hours the color of the serum changes from orange to deep yellow (bilirubinemia), and this color fades within 24 to 48 hours. These changes may occur in the absence of any untoward symptoms.

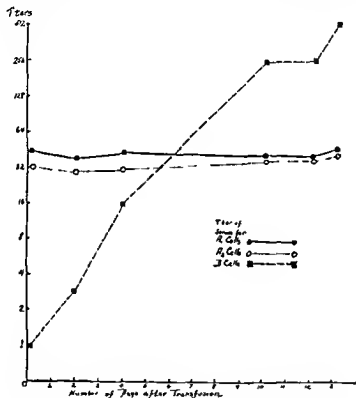


FIG. 36 CHANGES IN ISOAGGLUTININ TITERS OF THE SERUM OF A GROUP O INDIVIDUAL RESULTING FROM THE TRANSFUSION OF GROUP B BLOOD

It may be worth-while to examine smears of the patient's blood at the height of the reaction for evidence of erythrophagocytosis. Hopkins¹⁶ observed in blood smears made immediately after a transfusion of incompatible blood that the polymorphonuclear neutrophilic leucocytes contained an average of one to six phagocytosed erythrocytes. Ottenberg and Kaliski¹⁷ were able to demonstrate phagocytosis of the

¹⁶ *Arch Int Med* 6: 270 (1910)

¹⁷ *Jour Amer Med Assoc* 61: 2138 (1913)

red blood cells in 3 patients given transfusions of incompatible blood

As has already been pointed out, urine passed after the transfusion of incompatible blood is smoky red in color due to the presence of hemoglobin. According to Oehlecker, hemoglobinuria does not occur unless at least 60 to 80 c c of blood is hemolyzed, and recent work has shown that the concentration of free hemoglobin in the plasma must exceed a certain level before any passes through the kidney. It is important to examine the first urine sample passed after the transfusion, since hemoglobin is often present only in this single sample. The coincidental presence of blood in the urine due to metrorrhagia or hematuria should cause no confusion because in these conditions numerous intact red cells will be found in the urine and anuria does not develop.

When a reaction occurs, the samples of blood taken from the patient and donor before the transfusion should be retested. At most hospitals these samples are preserved in the refrigerator for 3 or 4 days after the transfusion for just this purpose. It may also be wise to test fresh blood samples to rule out any accidental interchange. If the blood samples have been discarded and fresh samples cannot be obtained, indirect evidence may be obtained by titrating the isoagglutinins in the patient's serum. For example, in a group O patient if the titer of the anti B agglutinin remains at about an average level after the transfusion, but the anti A agglutinin has a very low titer immediately after the transfusion but rises to a very high level within a week or ten days, this would indicate that the group O patient had received group A blood. As an example, figure 36 can be consulted, in which are charted the changes in isoagglutinin titer resulting from the transfusion of 500 c c of group B blood into group O individual.¹⁸ The titer of the anti A agglutinin remained practically constant at approximately 32, throughout the period of observation. On the other hand, the titer of the anti B isoagglutinin was only 1, two hours after the transfusion, and rose to 512 on the 13th day following the transfusion.¹⁹ The exceptionally low titer of the anti B agglutinin immediately after the transfusion was evidently caused by absorption of the isoagglutinin by the injected B blood, and the subsequent rise to the exceptionally high level of 512 represents in all probability an immune response to the foreign blood.

Hemolysis due to intragroup incompatibility offers somewhat greater

¹⁸ Incidentally aside from the initial chill, fever, hemoglobinemia and hemoglobinuria, this patient apparently suffered no serious effects from the transfusion of incompatible blood.

¹⁹ In a second case seen by the present writer where a group A patient was given 300 c c of group AB blood, a similar rise in the titer of the β isoagglutinin was observed. Also see *Rq Acta chir scand* 80: 283 (1938).

difficulties in diagnosis.²⁰ If the patient and donor belong to different M N types, it may be possible to demonstrate that all the donor's blood has been eliminated from the patient's circulation and in this way to establish a diagnosis. On the other hand, if there is no evidence of disappearance of the donor's blood, one is not dealing with a hemolytic transfusion reaction. If patient and donor belong to the same M N type it is still possible to arrive at a decision in cases where universal donor blood has been used for patients of group A, B or AB and possibly where patient and donor both belong to group A or group AB but to different subgroups. Where the above tests prove that hemolysis of the donor's blood has occurred, an attempt should be made to demonstrate the presence of irregular agglutinins in the patient's serum and to determine their nature. Where the patient is found to be Rh negative and the donor Rh positive anti Rh isoantibodies will not seldom be found in the patient's serum.²¹ If the tests on the pre-transfusion sample are inconclusive they should be repeated on samples drawn 5 to 10 days after the transfusion. Tests on blood drawn immediately after the transfusion are likely to be uninformative because of the so called negative phase.

Pathogenesis The exact mechanism responsible for the symptoms of hemolytic reactions is not completely understood. The incompatible red blood cells are apparently disposed of in part by erythrophagocytosis²² but in the acute cases destruction of the donor's blood cells results mainly from the direct hemolytic action of the circulating isoantibodies. As already mentioned when such circulating isoantibodies are of very high titer it has been assumed that agglutination of the donor's cells with embolization may result.

It is now generally accepted that the renal symptoms are caused by the hemoglobin released from the donor's broken down erythrocytes. A similar syndrome has been observed occasionally in cases of paroxysmal hemoglobinuria,²³ blackwater fever,²⁴ and acute hemolytic anemia from sulfapyridine²⁵ and quinine²⁶ therapy. As Fairley²⁷ has pointed out extracorporeal hemoglobin even from an individual's

²⁰ Wiener and Peters *Ann Int Med* 13: 2306 (1940); Wiener *Arch Path* 32: 22 (1941); Wiener *Amer Jour Clin Path* 12: 189 (1942).

²¹ Probably through the hemotropic action of the isoantibodies. cf. Hektoen *Jour Inf Dis* 3: 721 (1926).

²² Unpublished observations.

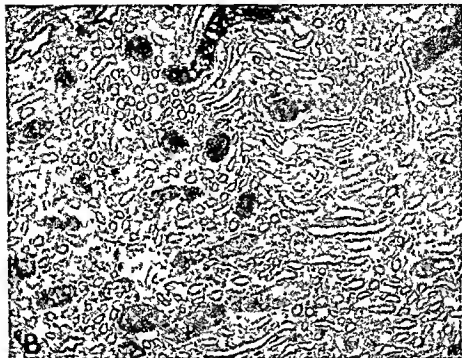
²³ Yorke and Naus *Ann Trop Med* 5: 287 (1911-1912); Dodge *Am Jour Hyg* 19: 208 (1920).

²⁴ Ravil and Chesner *Amer Jour Med Sci* 119: 380 (1940).

²⁵ Terplan and Gavert *Jour Amer Med Assoc* 106: 529 (1936).

²⁶ *Brit Med Jour* 2: 713 (1940).

own blood cells is treated as a foreign substance by the body and is disposed of very promptly. Part of the hemoglobin is absorbed by the reticuloendothelial system and part is broken down in the blood stream to methemoglobin and other hemoglobin derivatives. When the concentration of hemoglobin in the blood exceeds a certain level, the



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FIG. 37. PHOTOMICROGRAPH OF A SECTION OF A KIDNEY IN A HEMOLYTIC REACTION (After Goldring and Graef)

This demonstrates pigmented granular masses obstructing the collecting tubules

hemoglobin passes out through the kidneys and in this process the kidneys may be damaged.

As to the mechanism by which damage results, two main explanations have been suggested. (1) The tubules are mechanically blocked by acid hematin interfering with the excretory function of the kidney. (2) The hemoglobin and other noxious products released by the hemolytic reaction have a direct toxic action on the renal parenchyma and also cause a spasm of the renal artery with resulting reduced filtration pressure.

The theory of mechanical blockage probably dates back to Ponfick.²⁷

²⁷ Virchow's Arch. f. path. Anat. 62: 273 (1875)

who observed the presence of blood pigment casts in the kidneys of dogs transfused with blood from other animal species. In studies on experimental hemoglobinuria in animals, Yorke and Nauss,⁸ Baker and Dodds,²⁹ and DeGowin, Osterhagen and Andersch³⁰ noted the importance of the urinary pH on the outcome of the transfusion. Baker and Dodds stated that when the urine is alkaline, the hemoglobin remains in solution and passes out through the kidney without damaging it, while when the urine is acid the hemoglobin is converted into acid hematin which blocks the tubules. However, the occurrence of liver necroses in hemolytic transfusion reactions, blackwater fever etc., point to some toxic action of the hemoglobin or some other substance released by the hemolytic process. If, as Hesse and Filatov,³¹ suggest, these noxious products released by the hemolytic reaction also cause a spasm of the renal artery, not only could this produce a reduced filtration pressure but it might also account for the sudden sharp lumbar pain experienced by the patient during the immediate reaction. Possibly both mechanisms play a rôle in damaging the kidneys and recent work appears to confirm the importance particularly of urinary pH in the outcome of hemolytic transfusion reactions.³²

Pathological Anatomy. In only comparatively few cases of death after the transfusion of incompatible blood have investigations been made on the pathological anatomical changes.³²

Postmortem examination of patients who died immediately after the transfusion may reveal multiple thromboses (including the capillaries). In patients who died in uremia, changes are seen in the kidneys. These are usually found to be swollen and the pyramids appear darker in color. Microscopically, there are degenerative changes in the tubular epithelium said to resemble those seen in mercurial poisoning and the tubules contain brownish pigmented material and pigment casts (cf fig. 37). Droplets of a similar material are found in the epithelial cells. In the interstitial tissue there is edema and cellular infiltration.

In a number of cases necrotic foci were seen in the liver on microscopic examination. Lindau has described severe hemorrhagic and ulcerative changes in the colon which would account for the occasional occurrence of bloody diarrhea in patient

⁸ *Ann Trop Med* 5: 287 (1911).

²⁹ *Brit Jour Exp Path* 6: 24 (1925). Baker *Lancet* 1: 1390 (1937).

³⁰ *Arch Int Med* 59: 432 (1937). Also see DeGowin, Warner and Randall *Arch Int Med* 61: 609 (1938).

³¹ *Zentrabl f Chir* 59: 2674 (1932).

³² However cf DeNavasquez *Jour Path and Bact* 51: 413 (1940).

³³ Lindau *Acta path scand* 5: 382 (1928). Baker and Dodds *loc cit*. Bordley *loc cit*. Lemke *Virchows Arch* 257: 415 (1925). Goldring and Graef *Arch Int Med* 58: 825 (1936), etc. Also see DeGowin, Warren and Randall *Arch Int Med* 61: 609 (1938).

suffering from hemolytic reactions. In other organs pathological changes have been noticed at times for example pin point hemorrhages in the serous membranes and in the kidneys and liver

Prognosis and Treatment As has already been mentioned, the transfusion of incompatible blood is not always followed by severe hemolytic reactions. With mild reactions, such as chills and fever without urinary suppression, recovery is the rule. However, once a typical hemolytic reaction with uremia sets in, whether due to intra or extra group incompatibility of the blood, the prognosis is grave. In Bordley's series of 17 cases, mostly compiled from the literature, 11 patients died, 3 out of 7 patients died in the series reported by Goldring and Graef. The height of the uremia occurs in the 8th to 12th day, death may occur between the 4th and 18th day. In most cases, uremia is the cause of death, but in a few instances³³ death was due to exsanguination resulting from the hemorrhagic tendency that developed immediately after transfusion.

Treatment should be directed to induce the onset of diuresis. For this reason intravenous injections of dextrose and saline solutions may be beneficial. In a case reported by Bancroft,³⁴ decapsulation of the kidneys was performed when the patient's condition seemed desperate, and this was followed by marked diuresis and gradual recovery. Treatment with alkalis has been advocated by Baker and Dodds,³⁵ based upon their concept of the pathogenesis of the condition. Sodium bicarbonate in doses of 10 grains four times daily is well tolerated by mouth and usually sufficient to keep the urine alkaline. Mandelbaum³⁶ points out that the use of larger doses may cause alkalosis in these patients, because of the lack of adjusting power of the kidneys.

Some authors advocate the routine administration of alkalis before and after each transfusion as a prophylactic measure. As an emergency procedure the intravenous injection of 100 c.c. of 3.8 per cent sodium citrate solution is recommended because of the ready availability of this solution. Others particularly Hesse and Filatov³⁷ recommend the immediate and repeated transfusion of certainly compatible blood. These authors claim excellent results, specially where blood of an incorrect group was originally transfused.

Reactions Following the Intramuscular Injection of Blood The intramuscular injection of whole blood has been suggested for instance for the prophylaxis of measles and the treatment of hemorrhagic disease of the newborn (cf. page 84). When blood is given by this route preliminary blood grouping is considered unnecessary. Still intramuscular injections of incompatible blood may give rise to re-

³³ Parr and Krischner *loc cit*, Abramson *Jour Lancet* 55: 803 (1935). Wiener and Peters *Ann Int Med* 13: 2306 (1940).

³⁴ *Ann Surg* 81: 733 (1925).

³⁵ *Brit Jour Exp Path* 6: 247 (1925).

³⁶ *Ann. Int Med.* 12: 1699 (1939).

³⁷ *Zentralbl f Chir* 59: 2674 (1932).

actions in a small percentage of cases, though without serious consequences

Such observations were made by Kaiser,²⁸ who injected 10 to 15 c.c. of blood intramuscularly into each of 214 children and noticed the development of symptoms in 5 children. Rapoport and Stokes²⁹ had very similar results in a much larger series. A typical reaction may be described as follows. After an incubation period varying between 2 and 10 days, there is a rise in temperature, at times as high as 104°F., associated with malaise and leucocytosis, and redness, swelling, heat and pain at the site of injection. These symptoms last from 2 to 7 days. In 17 such cases investigated, the cells of the donors were found to be incompatible with the sera of the recipients. This proves that when reactions occur, they are caused by the injection of incompatible blood.

MINOR REACTIONS FOLLOWING BLOOD TRANSFUSION

With present-day technic, hemolytic reactions are rare; on the other hand, minor reactions (chills and fever) are not infrequently encountered. The latter are far less serious. Practically all the minor reactions are due to non-specific agents, but a small percentage can be traced to incompatibility between the bloods of donor and recipient (cf. page 127). The incidence of reactions following transfusion ranges from 1 to 30 per cent or higher, varying greatly in the reports of different operators. Since, as can be concluded from the following discussions, most of these minor reactions are preventable, a high incidence (about 5 per cent) is a reflection on the method of transfusion.

When a rigor occurs following a transfusion, steps should be taken immediately to determine its cause, in order to prevent the occurrence of further possibly more serious reactions in the same and other patients. It is most important first to exclude errors in blood grouping or intragroup incompatibility, since a subsequent transfusion to the same patient could in such instances give rise to dangerous hemolytic reactions. After excluding incompatibility one should then consider the following causative agents: incipient coagulative changes in the blood (or frank clotting), and probably most important—the presence of foreign matter, in particular so called “pyrogens,” in the apparatus or solutions. While for a long time citrate was considered to play an important part in post-transfusion reactions, as was pointed out on page 95, recent work has proved it to be harmless even when relatively large doses are administered.

Foreign Matter Derived from the Apparatus. Some of the reactions to blood transfusion can be attributed to the accumulation of blood clots or of debris, which remain in the apparatus and needles after sterilization. Proper cleansing of the apparatus as outlined on page 131 helps to prevent such accidents, and it is advisable to flush the apparatus with saline immediately before use.

²⁸ *N. Y. State Jour. of Med.* 33: 521 (1933).

²⁹ *Amer. Jour. Dis. Child.* 53: 471 (1937).

Soluble toxic substances present in new rubber tubing may cause violent and even alarming though almost invariably not fatal, symptoms (cf fig 38) Such reactions can be prevented by soaking new

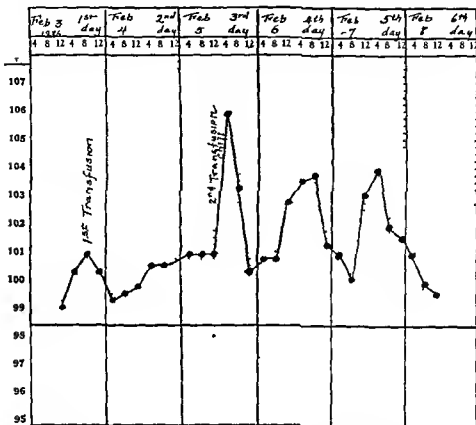


FIG 38 SEVERE CHILL AND FEVER FOLLOWING BLOOD TRANSFUSION

The patient was transfused to combat anemia caused by a bleeding duodenal ulcer. Following the first transfusion there were no untoward symptoms but one half hour after the second transfusion the patient had a severe chill lasting 30 minutes. The temperature rose abruptly from 101°F to 106°F. On investigation it was found that there had been chills and fever following other blood transfusions performed on the same day though not quite as severe and that on the day in question a new lot of rubber tubing had been put into use. There were secondary rises in temperature for two days following the transfusion reaction but thereafter the temperature was normal. Despite the reaction the hemoglobin rose from 6.1 gms per 100 c.c. before the 1st transfusion to 10.1 gms per 100 c.c. after the 2nd transfusion. The bleeding ceased and the patient made an uneventful recovery.

rubber tubing in normal sodium hydroxide for six hours, being careful that all parts of the lumen are reached,¹⁰ and then rinsing with distilled

¹⁰ Busman *Jour Lab and Clin Med* 5: 693 (1920)

water Tubing which has been treated to remove the soluble toxic substances is available commercially

Reactions caused by the presence of foreign matter or by the use of new rubber tubing can be recognized because they tend to occur in batches They not infrequently result from a change in personnel when the duty of preparing the transfusion apparatus is assigned to an inexperienced person

Incipient Coagulative Changes Some reactions have been attributed to incipient coagulative changes in the transfused blood before it reaches the patient's circulation The occurrence of such changes might account in part for the greater coagulability of the patient's blood after transfusion Satterlee and Hooker¹¹ suggest that the blood altered by its passage through the transfusion apparatus undergoes coagulative changes after reaching the patient's circulation but that the changes are too minute to be detected by ordinary methods

Occurrences of this sort are prevented by carrying out transfusions of unmodified blood expeditiously and with the citrate method by the correct adjustment of the tourniquet on the donor's arms and the use of a large gauge needle properly inserted into the vein to insure a free flow of blood

Pyrogens The importance of so-called "pyrogens" substances probably of bacterial origin as a cause of febrile reactions following intravenous injections has been recognized for many years, but only recently has this knowledge been applied systematically for the prevention of transfusion reactions

The occurrence of chills following intravenous injections is a disturbing complication the cause of which was not understood for a long time These reactions were at first ascribed to chemical impurities in saline and glucose solutions or to the direct action of the drugs administered such as salvarsan Wechselsmann¹² showed however that when freshly distilled and bacteria free distilled water was used for preparing salvarsan solutions such reactions did not occur From supposedly sterile solutions which gave reactions on the other hand he isolated a gram negative spore bearing bacillus and he therefore postulated that the reactions were caused by contamination rather than the salvarsan itself Shortly thereafter Hart and Penfold¹³ showed that sterile chemically pure distilled water injected shortly after distillation does not produce fever but on standing substances named pyrogens by them appear in the water which are not removed by Berkefeld filtration These were believed to be of bacterial origin even though the centrifuged sediment of bacterial bodies when injected did not produce fever Hart and Penfold noted that some pyrogens gave rise to a late continuous fever others to an immediate fugitive fever

Seibert¹⁴ showed that intravenous solutions prepared from ordinary laboratory

¹¹ *Jour Amer Med Assoc* 66 618 (1916)

¹² *Munch med Woch* 8 1510 (1911)

¹³ *Brit Med Jour* 2 1589 (1911)

¹⁴ *Amer Jour Physiol* 67 90 (1923) 71 621 (1925)

distilled water usually gave febrile reactions. On the other hand she showed that impurities such as silicates dissolved from containers or particles of cork do not cause such reactions. Tap water during certain months was found to contain pyrogens while at other times it caused no febrile reactions. From pyrogenic tap water Seibert isolated a small rod like gram negative bacillus and showed that filtrates of cultures of this bacillus in relatively minute doses were capable of causing severe febrile reactions. The substance was relatively heat stable being destroyed only by long heating. More recently Fui and Schrift⁴⁵ who studied cultures of a variety of pathogenic and non-pathogenic bacteria found that the capacity to produce pyrogenic substances is not confined to special organisms.

The observations on the rôle of pyrogens in febrile reactions to intravenous infusions are also applicable to blood transfusions. If the distilled water used for cleansing the apparatus and preparing the citrate and saline solutions is pure, such reactions can be prevented. Probably most of the reactions formerly ascribed to citrate were really caused by the distilled water used to dissolve it.⁴⁶

Pyrogen free distilled water can be obtained, as suggested by Seibert, by using an all Pyrex glass distilling apparatus with a spray catching trap. If the water is not used within a few hours, it should be autoclaved in order to sterilize it and then stored. All solutions must be prepared with such water and chemically pure reagents and sterilized within two hours.

Used apparatus should be disassembled and all parts mechanically cleansed, flushed with running tap water and then immediately rinsed with several changes of the pyrogen free distilled water in order to remove all traces of the tap water. The apparatus is then dried in air, reassembled and autoclaved within two or three hours in order to allow no opportunity for bacterial development in traces of water remaining in the apparatus. With new rubber tubing, as already mentioned the cleansing should be preceded by treatment with alkali, either soaking the tubing in alkali for 6 hours or autoclaving it in the alkali for 15 minutes. New glassware or glassware difficult to cleanse mechanically should be treated with sulfuric acid dichromate solution, and then cleansed as outlined above.

In this connection Seibert's observations that hemolysis per se, produced in experimental animals by injection of distilled water, does not cause rigors is in line with recent observations on stored blood. Earlier reports that blood more than 10 days old gave severe reactions can be ascribed to the use of an open technic of collecting blood, resulting in contamination which may become appreciable in amount after the blood has been stored for a while in the refrigerator. With the newer

⁴⁵ *Jour Lab and Clin Med* 27: 569 (1941)

⁴⁶ Cf. Wiener, Oremland, Hyman and Samwick *Amer Jour Clin Path* 11: 102 (1941)

closed methods of collecting blood no such correlation in the incidence of chills and the age of the blood has been observed, even though very old and obviously hemolyzed blood is transfused. As was pointed out previously, however, the use of old blood is objectionable on other grounds (cf. page 114).

REACTIONS OF ALLERGIC TYPE

Symptoms of varying severity, simulating allergic phenomena, are not infrequently observed after blood transfusions. Usually they consist merely in urticaria (and eosinophilia) and respond to small doses of adrenalin. Blottner⁴⁷ and Traum⁴⁸ have described instances of shock following repeated transfusions of compatible blood from the same donor.

Duker and Stofer⁴⁹ observed allergic symptoms in food sensitive persons receiving blood from donors who had ingested the offending food shortly before the transfusion. According to Brem,⁵⁰ such sequelae can be prevented by the use of fasting donors.

In this connection, a case reported by György and Witelsky⁵¹ is of interest. An 8 year old child (group O) had been transfused with group O blood several times: the father acting as donor on September 6th (200 c.c. of blood) and mother as donor on September 7th (130 c.c. of blood). When the child was again transfused with the father's blood on September 27th (200 c.c.) signs of severe anaphylactic shock appeared (The pulse became thready, there was a transient generalized erythema of the skin and edema of the face). Subsequently, it was demonstrated by means of skin tests that the child was sensitive to the father's serum though not sensitive to its own or the mother's serum. The child's serum also gave positive complement fixation reactions with the father's serum.

As György and Witelsky point out this might indicate that the child was hypersensitive to some individual specific substance in the father's serum but it could also be explained by assuming hypersensitivity to some foreign food circulating in the father's serum.

Related to the problem of allergic reactions to blood transfusion is the passive transfer of sensitiveness from donor to patient. Ramirez⁵² was the first to mention this possibility when he described the occurrence of horse asthma in a man who had been transfused two weeks previously. Holder and Diefenbach⁵³ reported the passive transfer from donor to patient of sensitiveness to strawberries, which is said to have persisted for three months. Garver⁵⁴ transfused three patients

⁴⁷ *Deutsche med. Woch.* 50: 599 (1924)

⁴⁸ *Deutsche Ztschr. f. Chir.* 237: 97 (1932)

⁴⁹ *Med. Clin. North America* 7: 1253 (1924)

⁵⁰ Brem, Zeiler, and Hammack, *Amer. Jour. Med. Sci.* 175: 96 (1928)

⁵¹ *Münch. med. Woch.*, p. 195 (1928)

⁵² *Jour. Amer. Med. Assoc.* 73: 984 (1919)

⁵³ *Calif. and West. Med.* 37: 387 (1932)

⁵⁴ *Jour. Allergy* 11: 32 (1939)

each with 500 c c of blood from donors with multiple sensitiveness and detected cutaneous sensitiveness in the patients to such antigens as spinach, horse dander, timothy weed and ragweed pollen as early as 14 hours after the transfusion, the reactions reaching their maximum by the fourth or fifth day and largely disappearing by the fourteenth day. In a similar study, using ragweed sensitive donors, Loveless³⁵ demonstrated the passive transfer of their hypersensitivity to recipients previously not allergic to pollen. Loveless showed that the sensitizing factor (reagin) in the transfused blood was quickly removed from the patient's circulation and was taken up by the skin, conjunctiva and nasal mucosa. The skin was found to be the first of these tissues to acquire sensitivity (as early as 1 to 2 hours after the transfusion) and last to relinquish it (sensitivity being demonstrable by skin tests for periods up to 3 months).

Probably many of the so called cases of allergic shock following transfusion are really instances of speed shock. In experiments on dogs Hirschfeld, Hyman and Wanger³⁶ showed that rapid intravenous injections of fluid gave rise within 40 to 60 seconds to cardiac failure, a sharp drop in the blood pressure and respiratory distress.

COMPLICATIONS OF TRANSFUSION

Aside from the reactions described, certain other disturbances and complications are occasionally observed following blood transfusions. Patients sometimes complain of severe headaches, dizziness, and a pounding sensation in the head. These symptoms usually are only of short duration but may last for several hours. Local pain in the arm may be complained of during the transfusion, caused by the distention of the vein wall as each syringeful of blood is injected. In infrequent instances, thrombosis of the punctured vein has been observed.

Cerebral hemorrhage or embolism and pulmonary embolism are rare complications. The latter is evidenced, usually by a dry, hacking cough, at times associated with pain in the chest or hemoptysis, either during the transfusion or soon thereafter. The danger of embolism is greatest in cases of subacute bacterial endocarditis and puerperal infections.

Whereas the transfusion of too large an amount of blood may lead to overburdening of the circulation and pulmonary edema, in the absence of cardiac disease the fairly rapid introduction of 500 c c of blood is well tolerated by the average adult patient. In this connection may be cited the experiments of Eyster,³⁷ who found that transfusions up to 1 per cent of the body weight resulted in only transitory alterations

³⁵ *Jour Immunol* 41: 15 (1941)

³⁶ *Arch Int Med* 47: 259 (1931)

³⁷ Cited after Polayes and Lederer *Jour Lab and Clin Med* 17: 1029 (1932)

of the cardiac size. However, it is doubtful whether a diseased heart would show a similar compensatory ability and, in fact, Eisenberger⁵⁸ has reported an instance of death from sudden cardiac paralysis in a patient with mitral stenosis which followed the injection of only 150 c c of blood. Probably a number of the other cases of sudden death following transfusion which have been reported in the literature can be explained in this way. This serves to emphasize the advice already given to inject the blood slowly, by the gravity method, in all patients with cardiac disease or poor myocardial tone.

The impression that transfusions are particularly dangerous if given while the patient is under the influence of an anesthetic does not seem justified. There is only the almost negligible danger of overlooking symptoms due to incompatibility. Cashman and Baker⁵⁹ observed 8 chills (6.4 per cent) among 125 transfusions on patients not under anesthesia, but not a single chill among 121 transfusions performed during operations.

While air embolism was an occasional complication of blood transfusion at one time, it is generally assumed that with modern technique this danger is completely eliminated. However, Simpson⁶⁰ has recently reported five air accidents occurring during transfusions. In one case the mere insertion of a cannula into the antecubital vein permitted enough air to be introduced to kill the patient. To avoid this complication it is recommended that during the infusion of the blood the clamp used to regulate the rate of flow be placed only a short distance above the recipient needle instead of between the drip regulator and the reservoir containing the blood.

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DIAMOND. Hemolytic Transfusion Reactions due to the Rh Factor. A Preventable Danger. *New Eng Jour Med* 227: 857 (1942).

⁵⁸ Cited after Polayes and Lederer *loc cit*.

⁵⁹ *Amer Jour Obst and Gyn* 31: 240 (1939).

⁶⁰ *Lancet* 1: 697 (1942).

CHAPTER IX

TRANSFUSION OF STORED BLOOD, PLASMA AND SERUM

WHEN AN indication for blood transfusion arises the best results will usually be obtained with fresh blood. However, depending on circumstances, the use of stored blood has its advantages. In civil life blood may be urgently required in some small outlying community, in which no suitable blood donor can be found. The citrate method lends itself admirably to the solution of such problems, since the donor's blood can be drawn into citrate solution and transported to the patient's home. Situations of this sort arise most frequently in war time, and the first extended experiments with stored blood were made during World War I. Robertson¹ used the method of preservation recommended by Rous and Turner,² mixing 3 parts of blood with 2 parts of 3.8 per cent sodium citrate solution and 5 parts of 5.4 per cent glucose solution. Because of the large bulk of the mixture and the large amount of citrate, the supernatant was siphoned off before the transfusion and the cells were resuspended in physiologic saline containing a small amount of added gelatin. Robertson states that he used blood stored as long as 26 days by this method and among 22 transfusions encountered only a single chill.

The transfusion of stored blood was only resorted to sporadically after the war, until its use was revived by the investigators Filatov,³ Bagdassarov, etc., in Russia several years ago. The use of stored blood was introduced into the U. S. A. at the Cook County Hospital, where the "blood bank" idea was originated.⁴ This was particularly attractive because of the large financial saving made possible by the use of blood banks, the need for professional donors being almost entirely eliminated. By the end of 1939, more than 15,000 transfusions of stored blood had been given in this country, and the number of such transfusions has been increased enormously since that time by the wide spread formation of blood banks, particularly in municipal state or county charitable hospitals, both in this country⁵ and abroad.

¹ *Brit Med Jour* 1: 691 (1918); *Jour Amer Med Assoc* 114: 520 (1940).

² *Jour Exp Med* 23: 219, 239 (1916).

³ Filatov, *Ist Khir* (Russian) 51: 194 (1937), abstracted in *Jour Amer Med Assoc* 109: 1410 (1937).

⁴ Fantus, *Jour Amer Med Assoc* 109: 128 (1937); *ibid* 111: 317 (1938).

⁵ Giddings and Kruger, *Hospitals* 13: 41 (March 1939); Cameron and Ferguson, *Surgery* 5: 237 (1939), etc.

Advantages of Blood Banks One of the principal advantages of blood banks is their convenience. As soon as the need for a transfusion arises blood of the proper group is obtained from the bank, and this is replaced later on with blood taken from volunteer donors, regardless of their groups, provided by the patient's family. The collection of blood, the grouping and Wassermann or flocculation tests can be carried out more conveniently by taking several donors at one time. From the patient's standpoint, the existence of a blood bank at a hospital is a safeguard in emergencies and during major operations, since blood of the proper group is always at hand when needed.

If more than one relative or friend appears at the blood bank the usual procedure is to take blood from all of them, even though the patient is given only a single transfusion. This is necessary in order to maintain a positive bank balance, and to allow for possible waste and spoilage. In this way also blood becomes available for patients who have no friends or relatives that can act as donors. Thus the need for professional donors is practically eliminated except for certain rare cases (e.g., intragroup incompatibility, patients of group AB) and a large financial saving is possible.

Disadvantages and Limitations of Stored Blood for Transfusion Blood does not last indefinitely, even when stored in the refrigerator under sterile conditions. For reasons pointed out below, at most institutions the time limit set for the use of stored blood is ten days. In order to have a good stock of the less common groups, B and AB, about 50 containers of blood taken from random donors are required or sufficient for an average of 5 transfusions a day. However, if one resorts to the use of group O blood for group B patients and group A blood for AB patients whenever blood of the homologous group is not available, it is possible to get along with a much smaller number of units of blood and in this way blood banks can be set up in smaller hospitals.

If there is a sudden large demand for blood, the stock of blood may be depleted. On the other hand, a drop in the demand for blood may leave the bank with a lot of old blood which can no longer be used for transfusion. Formerly this blood was wasted but with the introduction of plasma banks a use has been found for this material (cf page 149).

For best results it is necessary to have a large refrigerator, the temperature being thermostatically controlled between 4°C and 6°C. This is the most expensive, but, at the same time, the most important equipment required. Blood stored at higher temperatures has been found to deteriorate more rapidly, on the other hand, very low temperatures must be avoided as freezing the blood results in destruction of the red cells.

Investigations on the fate of the transfused stored erythrocytes in the patient's circulation have been carried out by Wiener and Schaefer,^{3a} using anti-M and anti-N agglutinating sera (cf. page 75). These investigators found that while fresh blood or blood only a few days old survived for periods up to three or four months, older blood

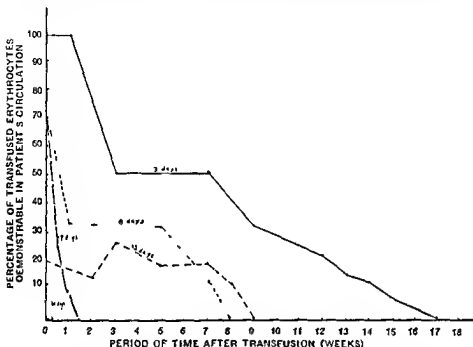


FIG 39 RESULTS OF TRANSFUSIONS OF BANK BLOOD

Percentage of donor's blood cells surviving at varying periods following transfusion [Five illustrative cases. Period of storage (in days) of blood used for transfusion is indicated on each curve]

[From *Med Clin North Amer* 24: 705 (1940)]

disappeared from the patient's circulation sooner, for instance, blood stored as long as 21 days was found to survive only 24 hours (cf. fig 39). Similar results have been obtained by Levine⁶ and Belk and Barnes.⁷ Moreover, Wiener and Schaefer found that while transfusions of blood less than 8 days old were not followed by noticeable bilirubinemia, this regularly occurred, at times together with hemoglo-

^{3a} *Med Clin N Amer* 24: 705 (1940), *Quarterly Bull Sea View Hospital* 5: 17 (1939). These studies were carried out on blood mixed with citrate solution and with no glucose.

⁶ Personal communication.

⁷ *Amer Jour. Med Sci* 201: 838 (1941).

binemia and hemoglobinuria, in patients receiving older blood.* In view of these findings, blood stored for periods longer than a week may be expected to give inferior results in the treatment of anemias, and fresher blood would be preferable.

As has already been pointed out, Robertson, in the first transfusions in man of stored blood, kept it in a mixture of citrate and glucose solutions. For simplicity, however, in most blood banks, the blood is merely mixed with citrate solution. Because hemolysis visible to the naked eye sets in later when glucose is added, DeGowin et al.⁹ assert that the addition of glucose makes blood fit for use for periods up to 30 days while blood kept in citrate alone should be discarded after 10 days. However, these authors have made no experiments to determine the survival in the patient's circulation of transfused blood cells stored for such long periods of time, and from *in vitro* tests Belk and Rosenstein¹⁰ believe the period of storage should not be prolonged beyond 14 days even when glucose is added as a preservative.^{10a} In this connection, Rous and Turner's observations in rabbits on the fate after transfusion of rabbit cells stored in saccharose citrate solution are of significance. These workers found that blood stored up to 14 days functioned normally after transfusion while blood kept for longer periods, though intact and apparently unchanged when transfused disappeared from the circulation within a few days though this was unaccompanied by any signs of hemolysis or any other symptoms. Autopsy of rabbits receiving blood 14 days old and older revealed hemosiderin deposition and erythrophagocytosis by the endothelial cells in the spleen.

The changes which occur *in vitro* when blood is stored have been the subject of a number of investigations. Bagdassarov¹¹ found that in most media (sodium citrate solution, glucose, etc.) there is a progressive increase in the fragility of the red cells even when stored under sterile conditions at the optimal temperature, and at the end of 15 days distinct hemolytic changes are noticeable.¹² It has also been found

* Cf. Brewer, Maizels, Oliver and Vaughn *Brit. Med. Jour.* 2: 48 (1940).

⁹ DeGowin, Harris and Plass *Jour. Amer. Med. Assoc.* 114: 850 (1940).

¹⁰ *Amer. Jour. Med. Sci.* 201: 841 (1941).

^{10a} In a recent study, Morrison and Young [*Brit. Med. Jour.* 2: 197 (1941)] found that red blood cells stored in dextrose citrate mixtures persist longer in the recipient's circulation than erythrocytes stored in citrate alone, confirming the conclusions drawn from *in vitro* studies. Cf. DeGowin, Harris and Bell *Proc. Soc. Exp. Biol. and Med.* 49: 481 (1942); DeGowin, Harris, Bell and Hardin *ibid.* 49: 484 (1942).

¹¹ *Le Sang* 11: 466 (1937). Cf. Kolmer *Amer. Jour. Med. Sci.* 197: 442 (1939).

Belk, Henry and Rosenstein *ibid.* 198: 631 (1939).

¹² Defibrinated blood was found to be much more labile than citrated blood, probably because of the mechanical injury incidental to defibrination. For this and other reasons (cf. page 146) the use of defibrinated blood is not recommended.

that the platelets, fibrinogen and leucocytes disintegrate rapidly,¹³ and within 24 hours the prothrombin time is so greatly prolonged¹⁴ that the blood is practically useless as a hemostatic agent in hemorrhagic diseases such as hemophilia and purpura. In infections, one would expect as good results from bank blood as from fresh blood, considering the stability of natural and immune antibodies when stored *in vitro*. Thus, for the prophylaxis of infectious diseases such as measles, stored or convalescent serum seems to be as effective as fresh whole blood or serum. A marked shift of potassium ions from the erythrocytes has been reported by Scudder,¹⁵ but in transfusions of stored blood by the usual gravity method this should not give rise to toxic symptoms.

Finally, it must be mentioned that when stored blood is used, rigid precautions as to sterility are necessary, because even a few microorganisms may multiply into large numbers over a period of time. For this reason, closed methods of collecting the blood are now used by almost all blood banks. Even with rigid precautions, however, some samples are bound to be infected, and while the contaminating bacteria or fungi are not likely to be pathogenic they can give rise to severe chills and fever following transfusion. Bacteria can also break down or damage the red cells before the usual expiration date and thus give rise to hemolytic reactions. Routine bacteriological examination is usually not carried out, however, because it is laborious, and the mere procedure of collecting a sample for culture may lead to contamination.

Jorda¹⁶ states that an automatic bacteriological control is possible by converting the reduced hemoglobin in the stored blood into oxyhemoglobin. This he accomplished by storing the blood under a pressure of two atmospheres of filtered air. According to Jorda, accidentally contaminated blood changes color from ruby red to dark red or black as the bacteria multiply. A simpler procedure, suggested by Diggs and Keith,¹⁷ is to mix the blood gently before use, then centrifuge a small sample in a test tube and inspect the supernatant for hemolysis. In this way free hemoglobin trapped in the sediment among the red cells can be detected. The addition to the stored blood of sulfanilamide,¹⁸ methylene blue,¹⁹ and other drugs as preservatives has been suggested, but this idea has not been generally adopted because concentrations sufficiently high to act as a preservative are likely to cause hemolysis.

Reactions to Transfusions of Stored Blood. The reports in the literature as to the relative incidence of reactions after transfusions of fresh

¹³ Scudder, *Bull N Y Acad Med* 17: 373 (1941), Cf Kolmer, *loc cit*

¹⁴ Rhoads and Panzer, *Jour Amer Med Assoc* 112: 309 (1939)

¹⁵ *Jour. Amer Med Assoc* 112: 2263 (1939)

¹⁶ *Rev Sauid Guerra* 1: 329 (1937), *Lancet* 1: 773 (1939)

¹⁷ *Amer Jour Clin Path* 9: 591 (1939)

¹⁸ Novak, *Jour Amer Med Assoc* 113: 2227 (1939)

¹⁹ Spivak and Pelishenko, *Eksper. Med Kharkov* 1: 21 (1940). These workers added methylene blue to concentration 0.4 gms/100 cc blood

and stored blood are conflicting. For example, at the Cook County Hospital the expiration date was set at 10 days because an increase in the number of reactions was observed with blood kept for longer periods.²⁰ On the other hand, no difference in the incidence of chills after fresh and stored blood transfusions was observed by DeGowin and Hardin² in a series of 1600 transfusions, or by Hoxworth and Skinner²² in a series of 3077 transfusions even when blood stored for periods up to 30 days was used. The average incidence of chills and fever in both these series was 4.1 per cent. Finally, Muether and Andrews² had a paradoxically lower incidence of chills following transfusions of stored blood (18 or 5.3 per cent among 337 transfusions) than of fresh blood (42 or 24 per cent among 169 transfusions).

The reasons for the discrepancies between these reports become evident when one considers the multiplicity of agents that can cause chills and fever after transfusions. The incidence of chills after transfusions of *fresh* blood varies among different workers from 1 to 30 per cent depending on the care used in cleansing the apparatus and preparing the solutions. The low incidence of reactions in the series of Hoxworth and Skinner and of DeGowin and Hardin can be explained by the scrupulous care they exerted over their transfusion equipment. With regard to reports of a sharp increase in transfusion reactions after 10 days storage, this may be due to the fact that the technic was still in the process of development in the earlier days of the blood bank, and possible infection of the blood with bacteria and fungi was responsible.²⁴ This seems plausible since with longer periods of storage and multiplication of the microorganisms, more violent reactions would be expected to result. For avoiding other potential sources of reactions, namely disintegrated leucocytes and platelets and precipitated fibrin, the use of transfusion apparatus having a stainless steel filter drip attachment (cf. fig. 44) has been recommended.

Since hemolysis is usually detectable in blood more than 14 days old and the red cells in blood stored even for shorter periods are noticeably more fragile, it may be difficult to understand why chills are not more frequent when old blood is transfused. It seems, however, that a certain amount of hemolyzed blood can be injected without any obvious symptoms provided it is free from any contamination with

²⁰ Fantus, *Arch. Path.* 26: 160 (1938).

²¹ *Brit. Med. Jour.* 2: 1 (1940).

²² *Arch. Surg.* 42: 496 (1941).

²³ *Amer. Jour. Clin. Path.* 11: 321 (1941).

²⁴ This appears to be the only reasonable explanation for the high incidence of reactions reported by Bagdasarian (65 per cent among a series of 20 transfusions) in contrast to the low incidence reported more recently.

bacteria or chemicals. With transfusions of stored blood containing larger amounts of free hemoglobin, DeGowin and Hardin observed hemoglobinuria of one or two days' duration but no other symptoms, and Muether and Andrews report 13 transfusions of blood 30 to 90 days old (average 52 days), all most likely badly hemolyzed, without a single chill. However, the present author does not approve the transfusion of blood kept for long periods because of the ever present danger of the development of a hemoglobin nephrosis, with resulting anuria and uremia (cf page 124). At some institutions, in fact, alkalies are routinely administered as a prophylactic before transfusions of stored blood in order to prevent precipitation of acid hematin in the kidney tubules. One reason why untoward results have not more frequently followed transfusions of old blood may be that the sodium citrate given with blood helps prevent this complication because it renders the urine alkaline.

Urticarial reactions apparently occur no more and possibly less frequently followed transfusions of stored blood than fresh blood. Hoxworth and Skinner noting this complication in about 1.5 per cent of their transfusions. As to the safety of stored blood for transfusion, Hoxworth and Skinner reported only two deaths in a series of 3077 transfusions, one of which probably had no relation to the transfusion, while in a series of 1600 transfusions reported by DeGowin and Hardin there were 2 deaths, neither due to the use of stored blood per se as one resulted from incompatible blood and the other from cardiac embarrassment. The reports of these operators therefore compare favorably with those on the transfusion of fresh blood (cf page 114).

Technic. As has already been pointed out, the blood should be collected with a closed system in order to reduce the chance of contamination with bacteria. Of the various methods suggested,²² two have proved most satisfactory. In both methods, a suitable container for the blood is required, preferably a graduated narrow bottle of hard glass. The size of the container selected should be such that with the proper amount of citrate or citrate glucose solution and blood, little dead space remains between the stopper and the upper surface of the blood mixture.

In the first method, a two hole rubber stopper is fitted onto the bottle, passing through one of the holes is a short glass tube connected to a rubber tubing (about 15 inches in length) having an adapter for an intravenous needle at the other end. In the other hole is a small tube with a cotton filter to act as an air vent. The author prefers to

²² Muether and Andrews *Amer Jour Clin Path* 11: 307 (1940), Boland, Craig and Jacobs *Lancet* 1: 388 (1939).

allow the blood to run with the aid of the venous pressure plus gravity but if the operator desires to use suction the glass tube used as an air vent can be connected to a suction bulb or suction mouthpiece by a piece of rubber tubing. A 15 gauge needle is attached to the adapter on the distal end of the rubber tubing and inserted into the donor's vein in the usual manner the collection of blood taking 3 to 10 minutes. The needle is then withdrawn and the two hole rubber stopper re

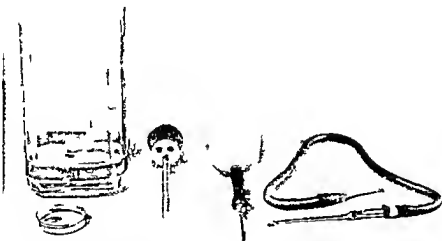


FIG. 403. EQUIPMENT USED FOR WITHDRAWING BLOOD FROM DONOR

Note the stopper with three or five holes; these or five do not go completely through the stopper.

(From Muether *Hospital Progress Bulletin* 163 October 1941)

placed by a solid one or better by a screw cap. In order to insure thorough mixture of the blood and citrate the bottle is gently rotated while the blood is being collected and for several minutes after the bottle is stoppered. The citrated blood is then placed in the refrigerator at once.

The second method has the advantage that it is unnecessary to change stoppers after the blood is drawn. The bottle used is similar to that used for the first method except that it is closed with a rubber diaphragm. When the citrate solution in the bottle is sterilized the

stopper is held in place only loosely, but as soon as the bottle is taken out of the autoclave, the stopper is forced firmly into place. As the bottle and solution cool, a partial vacuum develops. The only other equipment required is a piece of rubber tubing with adapters for needles at both ends. When the blood is collected, one needle is inserted into the donor's vein, the other is plunged through the rubber diaphragm. The blood will then run into the bottle, the rate of flow being regulated



FIG. 40b EQUIPMENT FOR DRAWING BLOOD ASSEMBLED

Needles perforate the rubber diaphragm over the orifices seen in figure

(From Muether *Hospital Progress*, Bulletin 163, October, 1941)

by a needle-valve or screw clamp. If through inadvertence the vacuum is lost before the full amount of blood is obtained, a needle to which is attached a glass adapter with cotton wool filter is plunged through the rubber diaphragm to act as an air-vent, and the blood is allowed to run by gravity. After the desired amount of blood has been collected, the needles are withdrawn first from the diaphragm, then from the vein, the blood in the tubing being collected in two "pilot" tubes, one dry, the other containing a little citrate solution. The pilot tubes are attached to the bottle, the blood in these tubes being used for the

grouping and serological tests. The bottle containing the blood is then covered with a screw cap and immediately placed in the refrigerator.

Many variations of these two major methods of drawing blood from donors can be devised. For example, the method just described for collecting blood by vacuum can be modified so that, instead of creating a vacuum in the receiving bottle as described above, a suction bulb, attached by adapter to a needle, is used (cf figs 40a and 40b). For the transfusion the blood is removed from the refrigerator and allowed to warm up to room temperature. If the blood is needed at once, there is no objection to administering it while cold, this being far safer than to attempt to warm the blood up to body temperature, since overheating can cause hemolysis. The simplest procedure is to pour the blood into a gravity apparatus and transfuse it by the drip method (cf page 98). An apparatus with a stainless steel filter (about 100 mesh to the inch) is desirable in order to remove any small clots, fibrin precipitate etc. that may have formed during storage. With the aid of a two hole rubber stopper, some glass tubing and rubber tubing an apparatus is readily contrived whereby the blood can be administered without removing it from the original bottle (cf fig 158). With blood collected by the second method, the screw cap is first removed then a long needle is plunged through the rubber diaphragm. This needle is to act as a vent, and its point should be above the level of the blood when the bottle is inverted. A rubber tubing with stainless steel filter drip and an adapter at each end is the only other equipment required. A short needle attached to the upper end of the tube is plunged through the diaphragm of the bottle which is then inverted and hung up on a stand. The tubing is allowed to fill with blood and the needle attached to its distal end is then inserted into the patient's vein.

Apparatus for either of the two methods described above completely assembled and ready for use, can be purchased on the open market.

At most blood banks the blood to be stored is merely mixed with citrate solution as for fresh blood transfusions. This procedure besides being more convenient, is most suitable where the blood bank is combined with a plasma bank. For more prolonged storage (cf page 138), modifications of the Rous-Turner preservative^{*} have been suggested containing less citrate solution than the original one, so that the entire mixture of blood and citrate-glucose solution can be safely transfused. The glucose and citrate solutions must be sterilized separately to avoid caramelization. DeGowin and Hardin mix every 500 c.c. of blood with 650 c.c. of 5.4 per cent anhydrous dextrose in water, and 100 c.c.

^{*}Perry *Wisc Med Jour* 25: 123 (1926). DeGowin and Hardin *loc cit*.

of 3.2 per cent dihydric sodium citrate in water. A disadvantage of this procedure is the large bulk of the mixture.

It is recommended that donors who provide blood for storage be subjected to the same examinations as donors for fresh blood transfusions. While it is assumed that the *Spirocheta pallida* is killed by five days' exposure to the cold of the refrigerator, still as a precaution at most blood banks routine serological tests are made on all donors²⁷ (cf. Fantus). Gordon²⁸ has reported the transmission of malaria to a patient as a result of the transfusion of infected stored blood. It might also be remarked that the use of bank blood increases the chances for clerical mistakes, so that particular pains must be taken in properly labelling the bottles and reading all labels before giving the transfusion. Mistakes have already occurred in which the wrong bottle of blood was used, and hemolytic reactions have resulted in this way.

Stored Blood in Military Medicine The use of stored blood has its greatest usefulness in war time. Since in male patients who have never been transfused previously, the first transfusion of blood of the homologous group has never been reported to have caused a hemolytic reaction (cf. page 67), the cross-match test is superfluous provided the grouping tests have been carried out accurately (testing both cells and serum), and clerical errors are excluded. Therefore, if the group of the wounded soldier is recorded on his identification tag and stored blood of the correct group is available, the transfusion can be given at once. In emergencies where the patient's group is not known, group O blood can be used. Since blood can be transported hundreds of miles within a few hours by airplane, the collection and storage of blood can be carried out at one or more central depots, and blood can be shipped to the field as required, not too large a reserve being maintained there in order to avoid waste. This plan is admirably supplemented by the use of stored plasma or serum, which because of its greater keeping qualities, particularly in the dried state, can be kept at hand at all times in much larger amounts.

TRANSFUSION OF PLASMA, SERUM AND OTHER BLOOD SUBSTITUTES

*History of Blood Substitutes.*²⁹ The present keen interest in blood substitutes is easy to understand, considering that in time of war there

²⁷ As an additional safeguard, Eichenlaub, Stolar and Wode [*Arch. Derm. and Syphilol.* 44: 441 (1941)] have suggested the addition of mapharsen to the citrate solution, 10 mg. for every 500 cc. of blood. Cf. Kast, Peterson and Kolmer, *Amer. Jour. Syph., Gon. and Ven. Dis.* 23: 150 (1940), McCluskie, *Lancet* 1: 264 (1939).

²⁸ *Jour. Amer. Med. Assoc.* 116: 1200 (1940).

²⁹ Cf. Amberson, *Biol. Rev.* 12: 43-86 (1937), Report of the Council on Pharmacy and Chemistry of the Amer. Med. Assoc., Human Blood Plasma and Serum, *Jour. Amer. Med. Assoc.* 117: 934 (1941).

is need for conservation of time and of blood. During the last few decades of the nineteenth century there was also some interest in blood substitutes but for quite a different reason. The use of citrate or other safe anticoagulants not being known at that time blood for transfusion was defibrinated by whipping it with egg beaters, broom bristles and the like. In view of this treatment and ignorance of the blood groups it is not surprising that these transfusions invariably resulted in severe chills and fever and with alarming frequency even caused the death of the patient instead of benefiting him. This led Thomas³⁰ to try transfusions of cow's milk as a substitute for human defibrinated blood. He decided to try this blood substitute because of the physical resemblance between milk and lymph and because Hodder in 1850 had successfully used milk injections in the treatment of Asiatic cholera administering as much as 14 ounces at a sitting. Moreover, Donne in animal experiments had injected milk into the veins of dogs and rabbits without ill effect. However, Schafer⁷ found that in depleted animals while recovery followed transfusions of defibrinated blood of the same species, if given transfusions of milk the animals succumbed.

Related to the question of blood substitutes are early perfusion experiments on isolated organs, in particular the heart. Using this technic Bowditch in 1871 made the first investigations on the biological properties of blood serum and plasma by perfusing frog's hearts with sheep and rabbit serum. At this point it may be well to say a word of caution concerning the danger of too literally translating to man the results of these and later experiments in animals on account of the existence of certain fundamental physiological differences among animal species and because as Wiggers³² has pointed out shock in experimental animals may be quite different from clinical shock. For example, by carefully washing out the blood from the bodies of frogs and replacing the blood by Ringer's solution (salt frogs (Salzfrosche)) have been obtained³³ which remain alive indefinitely despite the reduction of the red cell count to as low as 3000 to 6000/cu mm or about 1 per cent of the normal for frogs, a feat that cannot be approximated with warm blooded animals.

Among the first blood substitutes tried in the perfusion experiments performed during the last century were physiological saline, Ringer's and Locke's solutions. As early as 1878 Schafer remarked that in hemorrhage saline alone is of little value and that intravenous infusion

³⁰ Thomas *N Y Med Jour* 27: 449 (1883)

Trans Obst Soc London 21: 316 (1891880)

³² Wiggers *Jour Amer Med Assoc* 117: 1143 (1941)

³³ Gayda *Arch Sci Biol Napoli* 2: 333 (1921)

of such fluids should be unnecessary "for the half-filled blood vessels of the stomach would greedily take up from that organ any fluid that might be administered by mouth"³⁴ In hemorrhage, as we now know, the lost blood is replaced by tissue fluids, which can therefore be considered the natural or physiological blood substitute.³⁵ Tissue fluid approximates plasma in its composition, and it was soon found that plasma and serum contained some substance necessary for the maintenance of normal capillary permeability while glucose and salt solutions (so-called "crystalloid" solutions) were ineffective as blood substitutes because they quickly escaped from the blood vessels even when hypertonic solutions were used. The modern explanation for this property of serum and plasma is based principally on the work of Starling,³⁶ namely, that it depends on the purely physical properties of the serum proteins. In an attempt to improve his solution, Ringer³⁷ added gelatin to it, but this modification has not found favor because febrile reactions and even deaths have resulted from intravenous injections of gelatin.³⁸

At the beginning of this century, following the discovery of the blood groups by Landsteiner in 1900³⁹ and revival of interest in blood transfusion, the interest in blood substitutes diminished. The subject received new impetus as a result of World War I, which provided an opportunity for extensive clinical investigations. During the earlier part of the war postoperative shock and hemorrhage were treated with saline infusions, and while many lives were undoubtedly saved, in most cases the results were unsatisfactory because the rise in blood pressure was evanescent due to the escape of the fluid from the blood vessels into the tissues. In 1916, Bayliss³⁹ introduced the use of 6 per cent gum acacia in saline solution which he believed to be an ideal blood substitute, because of its supposed lack of toxicity, its possession of the same "viscosity" as blood and maintenance of the osmotic pressure due to the contained colloid. This solution proved to be far more efficient than the crystalloid solutions, and was used a good deal during the latter part of the war and after the war. Coburn⁴⁰ reported

"This interesting viewpoint has been the subject of a recent investigation by van Liere, Northrup and Vaughan [*Amer Jour Dig Dis* 7: 71 (1940)]. These authors found that in severe hemorrhage in dogs, saline solution placed in the intestines did not restore blood pressure even though it was absorbed while the same amounts of fluid given intravenously brought about distinct improvement.

³⁴ Robertson, *Jour Physiol* 84: 393 (1935).

³⁵ Starling, *Jour Physiol* 19: 312 (1896).

³⁶ *Jour Physiol* 6: 361 (1885).

³⁷ Steabben *Brit Jour Exp Path* 6: 1 (1925).

³⁸ Bayliss, *Intravenous Injection in Ill and Shock*, Longmann, Guent & Co. 1918.

³⁹ Coburn, *Jour Amer Med Assoc* 82: 1748 (1924), Coburn and Ward *Surg Clin N Amer* 5: 548 (1925).

the use of the solution postoperatively in 1000 cases without a single fatality. However, the pendulum soon swung the other way because it became evident that the results with gum acacia were definitely inferior to those with blood. "Mann" found that dogs suffering from experimental shock induced by exposure of the abdominal viscera responded satisfactorily to injections of dog blood or serum, but that only temporary improvement followed injections of gum acacia. Moreover, as pointed out by Amberson in his review, injections of gum acacia caused a marked increase in the sedimentation rate; the gum leaves the blood stream rapidly so that its effect lasts only 48 hours; and repeated injections of gum acacia resulted in a reduction of the plasma proteins possibly by blocking the reticulo-endothelial cells of the liver. On rare occasions anaphylactic reactions have resulted from repeated injections of the gum, perhaps due to protein impurities. Some of the disadvantages listed above may apply to certain blood substitutes recently suggested, such as isinglass³ and pectin.⁴

In recent years, particularly after it was recognized that whole blood is suitable for transfusion only if stored for a relatively short time, human blood plasma and serum have been used as blood substitutes. As has already been mentioned (cf. chapter VI), good results have been obtained with these blood substitutes in a number of conditions. Transfusions of unmodified animal sera, e.g., bovine sera, have been tried but are not recommended because of the high incidence of untoward reactions.⁵ Work is now in progress to obtain preparations of purified animal serum proteins for use as blood substitutes with the idea that such preparations will be less antigenic.⁶

The use of ascitic fluid was proposed by Davis and White⁷ in 1938, but thus far this material has only been used experimentally in animals. In conclusion, it should be pointed out that in none of the blood substitutes mentioned above is any attempt made to replace the lost red cells and their contained hemoglobin. Blood substitutes containing hemoglobin have been used with some measure of success in experimental

³ Keynes, *Blood Transfusion*, p. 35, Oxford Med. Pub. London (1922).

⁴ *Jour. Amer. Med. Assoc.* 71: 1184 (1918).

⁵ Taylor and Waters, *Canad. Med. Assoc. Jour.* 44: 547 (1941).

⁶ Hartmann et al., *Ann. Surg.* 114: 212 (1941).

⁷ Kremel et al., *Surgery*, 11: 333 (1942). The incidence of reactions was reduced somewhat by preliminary treatment of the bovine serum with human erythrocytes.

⁸ Cohn, *Chem. Rev.* 28: 395 (1941), Keys, Taylor and Savage, *Jour. Amer. Med. Assoc.* 117: 62 (1941), Taylor and Keys, *Proc. Soc. Exp. Biol. and Med.* 50: 325 (1942), Taylor, Keys and Savage, *ibid.* 50: 328 (1942).

⁹ Davis and White, *Proc. Soc. Exp. Biol. and Med.* 38: 462 (1938). Cf. Choissin and Ramsay, *Proc. Soc. Exp. Biol. and Med.* 38: 651 (1938).

animals, particularly by Amberson and his collaborators⁴⁸ but have thus far had very little clinical application because of the danger of renal complications (cf page 124) However, O Shaughnessy, Mansell and Slome⁴⁹ have reported the injection of 5 per cent hemoglobin in Ringer's solution, in amounts ranging from 200 to 1000 c c, in four patients with anemia, after preliminary alkalinization of the urine *Plasma vs Serum* In most of the earlier studies on blood substitutes, no distinction was made between plasma and serum In 1910, however, Maldovan⁵⁰ showed that injections into guinea pigs of strictly fresh defibrinated human blood or fresh human serum caused death with intracardiac and intravascular clotting This is due to the release of thrombin during the defibrination or clotting of blood and does not occur with serum that has been stored because of the spontaneous destruction of the thrombin However, later on substances appear in the serum which have a vasomotor action,⁵¹ while such substances are apparently not present in plasma

The sponsors of plasma⁵² in preference to serum as a blood substitute mention toxic reactions and even deaths which have followed injection of serum However, the proponents of serum transfusions⁵³ point out that when serum is properly prepared and free from particulate matter and aged, it is not toxic, and, furthermore, that serum is clearer than plasma and easier to sterilize by filtration Moreover, in plasma, after it has stood for a long period of time, a heavy precipitation of fibrin occurs which may resemble bacterial growth While such plasma can be transfused without harm if properly filtered, failure to filter the plasma may result in a serious reaction Incidentally, plasma from which the fibrin has been removed by filtration is really human serum diluted with citrate solution

Plasma transfusions are convenient at institutions which have blood banks, since the plasma can easily be obtained from blood not used before the expiration date of the blood is reached In that way plasma can be obtained as a by product from stored blood, while serum must be specially prepared As plasma obtained even from relatively fresh blood may contain small fibrin precipitates, it should be filtered before being administered⁵⁴ In order to combine the advantages of serum

⁴⁸ Amberson et al *Jour Cell and Comp Physiol* 5 359 (1934), Butte Kekwick and Schweitzer *Lancet* 2 507 (1940)

⁴⁹ *Lancet* 2 1068 (1939)

⁵⁰ *Deutsch med Woch* 36 2242 (1910)

⁵¹ For review of literature cf Amberson *Biol Rev* 12 48 (1937)

⁵² Strumia Wagner and Monaghan *Ann Surg* 3 623 (1941)

⁵³ Levinson Rubovits and Necheles *Jour Amer Med Assoc* 115 1163 (1940)

⁵⁴ Cf Maynard *Jour Amer Med Assoc* 115 2015 (1941)

with the ease of obtaining plasma from stored blood, Clegg and Dible³³ have advocated the immediate conversion of plasma into serum by the addition of calcium chloride. The serum obtained in this way remains clear even after prolonged storage and can be transfused through an ordinary intravenous set without filtration. In practice, it has been found that properly prepared human serum and plasma are equivalent in the clinical results they produce.³⁴

Advantages and Limitations of Serum and Plasma Transfusion It has been asserted that in plasma transfusions the group of the patient can be disregarded, so that the delay incidental to the grouping and matching tests can be eliminated. The consensus at the present time is that with plasma (or serum) prepared from individual samples of blood it is preferable to use only plasma of the same group as the patient with the exception that group O patients can receive any plasma and group AB plasma can be given to any patient. The common practice today is to eliminate the action of the isoagglutinins in the plasma by pooling. Edwards, Kay and Davie³ recommended pooling bloods of various groups before separating the plasma. This method is highly efficient because the red cells absorb the isoagglutinins in the plasma. Moreover, Hill⁴ points out that the agglutination of the red corpuscles aids the more complete separation of plasma, thus resulting in a larger plasma yield. The objection that the plasma obtained in this way is apt to contain hemoglobin as a result of isohemolysis is not serious as the amount of hemoglobin in properly prepared plasma will be too small to have any harmful effect. However, most workers prefer to pool the plasma (or serum) after its separation from the blood. Best results are obtained by pooling ten or more samples and with such pooled plasma the group of the patient can be safely disregarded. Pooling diminishes the titer of the isoagglutinins mainly by dilution and to some extent also through neutralization of the isoagglutinins by group substances in solution.³⁵ In emergencies where plasma prepared from only a single sample of blood is at hand this can be given safely regardless of the group provided that the plasma is run in slowly since foreign isoagglutinins when slowly introduced into the circulation are absorbed by antigens in the tissues (cf. chapter xvii) without any

³³ Clegg and Dible *Lancet* 2: 294 (1940)

³⁴ Levinson, Rubovits and Nacheles *Jour Amer Med Assoc* 114: 455 (1940)
Solandt *Canad Med Assoc Jour* 44: 189 (1941)

³⁵ *Brit Med Jour* 1: 317 (1940)

³⁶ *New York State Jour Med* 41: 1537 (1941)

³⁷ Levinson and Crenheim *Jour Amer Med Assoc* 114: 209² (1940). However
cf. page 18

harm resulting. In this connection, Witebsky et al.⁶⁰ have suggested the inactivation of the isoagglutinins in whole blood and plasma by the addition of purified solutions of group substances, and this would do away with the necessity for pooling plasma.

The greatest problem in relation to plasma and serum transfusion is the prevention of contamination by bacteria and fungi. While the multiplication of microorganisms is retarded in the refrigerator, plasma and serum permit bacterial multiplication, and after long periods of storage, even at low temperature, an initial slight infection may result in a significant growth. Routine bacteriological examinations and passage of the plasma or serum through bacterial filters do not necessarily guarantee sterility, since the handling the material receives may itself serve to introduce infection. The addition of preservatives such as merthiolate has been recommended by some workers, improper preservatives such as phenol are to be avoided as these precipitate the plasma proteins, and effective concentrations may prove toxic.

The difficulties entailed in the storage of liquid plasma are avoided by desiccation, in this way preventing bacterial growth. Hill⁶¹ states that the use of dried plasma also has the advantage that refrigeration is not essential, and the plasma can be kept at hand anywhere for immediate use. Moreover, the biologic properties are said to be preserved; fibrin precipitates do not form and the bulk of the material is reduced. Hill points out that by redissolving the dried plasma in a small volume of distilled water, for example one fourth the original volume, plasma is obtained in a concentrated form which can be administered intravenously simply by syringe. This concentrated plasma is said to have increased therapeutic potency producing a more rapid rise in blood volume in cases of shock⁶² and being useful for reducing intracranial pressure and for removing edema fluids, particularly in nephrosis.

As for reactions to plasma and serum transfusions, all recent reports agree that their frequency is low (ranging from less than 1 per cent to 3 per cent). The high incidence of reactions observed in earlier studies, such as those reported by Burceva (cf. table 12), was undoubtedly due to imperfections in the technic. Though many thousands of plasma and serum transfusions have been given to date, only a single fatality has been reported,⁶³ and in this case the technic employed was faulty since

⁶⁰ *Jour Amer Med Assoc* 116: 2654 (1941).

⁶¹ *Loc cit*.

⁶² Hill, Muirhead, Ashworth and Tigertt. *Jour Amer Med Assoc* 116: 393 (1941).

⁶³ Maynard. *Jour Amer Med Assoc* 116: 2015 (1941).

TABLE 12
INCIDENCE OF REACTIONS FOLLOWING PLASMA TRANSFUSIONS

Investigators	Technic of Storage and Transfusion	Number of Transfusions	Reactions
1 Butcova, <i>Arch f kl u Ch r</i> 182 710 (1935)	Liquid plasma stored for 10 to 165 days.	143	Reaction noted in 43 per cent of patients
2 Struma, Wagner and Monaghan <i>Jour Amer Med Assoc</i> 114 1337 (Apr 3) 1940	Liquid plasma with merthiolate 1:10,000 at 4°C for periods up to several months.	More than 1500	None. Rate of transfusion usually less than 10 c.c. per minute though gave as much as 900 c.c. in half hour without a reaction
3 Levinson, Rubovits and Necheles <i>Jour Amer Med Assoc</i> 115 1163 (Oct 5) 1940	Liquid serum stored in the refrigerator	4 patients	1 patient had local pain, swelling and redness which soon subsided.
4 Selfan (Scully) Report of the Blood Transfusion Association, Sept 15 1941	Serum stored mostly in liquid state in part also in dried state	156	41 or 26.2% Of these 5 were severe and infusion had to be stopped 26 were moderate (chills and fever) 6 were mild and 3 were questionable (probably due to other causes)
5 Elliot, Busby and Tatum <i>Jour Amer Med Assoc</i> 115 1006 (Sept 21) 1940	Citrated plasma diluted with equal volume of normal saline and with merthiolate 1:10,000 sealed stored in refrigerator or at room temperature for periods up to eighteen months.	482	Chills and fever
6 Mahoney, Kingsley and Howland <i>Ann Surg</i> 113 881 (June) 1941	Plasma dried in frozen state. Reconstituted with distilled water before transfusion	340 transfusions to 110 patients Max dose 800 c.c.	12 mild reactions (9 chills and fever 3 urticaria). One patient received 45 plasma transfusions.
7 Brown and Mollison <i>Br J Med</i> 2 819 (Dec 14) 1940	Serum dried in frozen state before transfusion and sealed in one-fourth or final volume of distilled water	91	febrile reactions 6, urticarial 2, urticarial reactions 3 etc etc but transient lumbar pain. All reactions mild. Frequent thromboses of veins occurred however and was attributed to use of 4 times concentrated plasma
8 Hill, Murhead, Ashworth and Tupper <i>Jour Amer Med Assoc</i> 116 395 (Feb 1) 1941	Plasma prepared from pooled citrated blood decanted in frozen state. Transfused in four times normal concentration in doses of 150 to 200 c.c.	229	3 mild febrile reactions. 1 patient developed pulmonary edema which was relieved by emesection. Occasional pain in vein but no thrombosis

the plasma was not filtered before the transfusion." Even plasma in four times its normal concentration gives rise to but few reactions on intravenous injection, the incidence of chills and fever in the hands of Hill and his co-workers being only 1 per cent. While Mahoney, Kings-

"Cf Lewis *Jour Amer Med Assoc* 116 2713 (1941) Wiener *Jour Amer Med Assoc* 116 2885 (1941)

ley and Howland reported the frequent occurrence of thrombosis of the injected vein when hypertonic plasma was injected Hill et al did not encounter this complication in a series of 229 transfusions though patients sometimes did complain of evanescent pain at the site of the injection

Technic of Preparing Liquid Plasma and Serum Plasma can be ob



FIG 41 WITHDRAWING PLASMA INTO POOLING BOTTLE

Note air filter water filter may be introduced into system if desired

(From Muether *Hospital Progress* Bulletin #163
October 1941)

tained as a by product from bank blood or specially prepared as in the project of the Blood Transfusion Association ⁴⁵ Blood for the preparation of plasma should be collected by a closed method under strictly sterile conditions Where the plasma is obtained from bank blood the simplest procedure is to remove the plasma by suction taking care not to disturb the sedimented red cells collecting the plasma from

⁴⁵ Report of the Blood Transfusion Association Concerning the Project for supplying Plasma for England Carried on jointly with the American Red Cross from August 1940 to January 1941 (Jan 31 1941)

10 or more bloods into a single large container (cf fig 41) A sample of the pooled plasma is removed for aerobic and anaerobic cultures and a preservative is then added If after one week no growth has occurred in the cultures, the pooled plasma is distributed among a number of individual containers for storage and subsequent use

Needless to say the transfer of plasma to the pooling flask must be done under strictly sterile conditions This is most readily accomplished in a special air

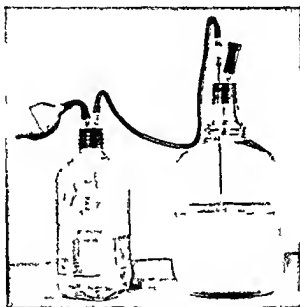


FIG. 42 EQUIPMENT SET UP FOR TRANSFER OF PLASMA TO FINAL CONTAINER

(From Muether *Hospital Progress Bulletin* #163
October 1941)

conditioned dust free room the operator wearing a cap and mask and using sterile apparatus with a few small pieces of sterile cotton as a filter in the tubing between pooling bottle and the suction apparatus and trap

The redistribution of the pooled sterile plasma is simplified by using a sterile piece of rubber tubing with a needle at each end (cf page 143) one to be pushed through the diaphragm of the pooling flask the other through the diaphragms of 500 c c bottles each containing 250 c c of sterile saline (cf fig 42) As a vent a second needle with a glass adapter and cotton filter is pushed through the diaphragm of the pooling bottle and the flow of plasma into the vacuum bottles is regulated with the aid of a needle valve or clamp this being closed entirely before starting each new bottle

The preservative selected for the Blood Transfusion Association's project was merthiolate (sodium ethyl mercury thiosalicylate Lilly) One c c of a solution containing 1 per cent merthiolate and 1.4 per cent borax was added for every 100

c.c. of plasma giving a final dilution of 1:10,000 of merthiolate. With regard to the toxicity of this preservative it is claimed that 50 c.c. of the 1 per cent solution has been injected intravenously into human beings with no effect and as much as 100 c.c. over a period of 5 days has proved harmless.

If cultures of the plasma are desired after the merthiolate has been added Meleney recommends that 1 c.c. of 1 per cent ammonium sulfate be added for every 10 c.c. of plasma and 50 c.c. of culture medium in order to precipitate the mercury.

As an additional safeguard and to rule out any toxic qualities in the plasma Strumia and McGraw⁶⁶ inject 1 c.c. of the pooled plasma intraperitoneally into each of two mice and observe them for 7 days.

When whole citrated blood stands in the refrigerator at 4°C, according to Strumia, McGraw and Reichel,⁶⁷ 30 per cent plasma separates in 1 day, 35 per cent in 2 days, 38 per cent in 3 days, 42 per cent in 5 days and 44 per cent in 7 days, while the yield on the same blood by centrifuging is 57.2 per cent. For this reason, many institutions prefer to separate the plasma by centrifuging instead of simple sedimentation. Strumia et al. use a centrifuge (No. 3 International, model F5), which can hold four 700 c.c. bottles, each containing 500 c.c. of blood. At many institutions, a centrifuge (No. 2 International) that can handle four 250 c.c. containers at a time is employed. Overheating the blood is prevented by the use of a special windshield to reduce the friction, and by air conditioning. When 250 c.c. centrifuge bottles are used the blood from each donor is divided between two dry centrifuge bottles for the preparation of serum, or two bottles each containing 25 c.c. of 4 per cent dihydric sodium citrate solution for the preparation of plasma.

To convert plasma into serum⁶⁸ (cf. page 150) 20 c.c. of 8 per cent sterile calcium chloride solution are added for each liter of pooled plasma with glass beads and the mixture is placed in a mechanical shaker. A firm clot separates and the supernatant fluid is passed through a Seitz filter and stored in sterile containers. The resulting fluid is clear like serum and remains so during storage and at the same time has the advantage of being obtained as a by-product of bank blood.

Dried Plasma and Serum. A variety of methods have been proposed for the preparation of desiccated plasma and serum.⁶⁹ A simple method employed by Edwards, Kay and Davie⁷⁰ is to dry the plasma under partial vacuum at body temperature. By this method, from every 100 c.c. of plasma they obtain 8 grams of a yellow amorphous product, which dissolved readily in warm water. Most workers prefer drying

⁶⁶ *Amer Jour Clin Path* 11: 288 (1941).

⁶⁷ *Amer Jour Clin Path* 11: 175 (1941).

⁶⁸ Clegg and Dible *Lancet* 2: 294 (1940).

⁶⁹ For a review cf. Harkins *Surgery* 9: 60-655 (1941).

⁷⁰ *Brit Med Jour* 1: 377 (1940).

the plasma in the frozen state, and a number of methods have been proposed for this purpose. In the lyophile method¹¹ the water vapor is removed by freezing, in the cryochem method chemical absorbents¹²

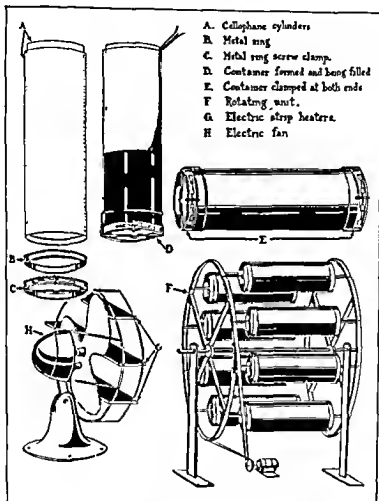


FIG 43 PLASMA DESICCATION APPARATUS

[From Hartman *Jour Amer Med Assoc* 115: 1989 (1940)]

are used, in the "desivac" method the water vapor is removed by suction,¹³ while in the "adtevac" method colloidal silica is used¹⁴ With the

¹¹ Florsdorf and Mudd, *Jour Immunol* 29: 389 (1935)

¹² Florsdorf and Mudd, *Jour Immunol* 34: 469 (1938)

¹³ Florsdorf, Stokes and Mudd, *Jour Amer Med Assoc* 115: 1095 (1940)

¹⁴ Hill and Pfeiffer, *Ann Int Med* 14: 201 (1940)

aid of a Tiselius apparatus, Scudder⁷⁵ has compared the electrophoretic patterns of fresh plasma, plasma dried under partial vacuum at 37°C, and plasma dried from the frozen state. While the latter differed but little from the original, plasma dried at body temperature showed wide variations, possibly indicating denaturation of the proteins. For this reason, and because of the 'flash' solubility of the product, plasma dried in the frozen state is to be preferred.

Of the other methods that have been suggested for desiccating plasma the one proposed by Hartman and Hartman⁷⁶ seems most practicable. The plasma is placed inside a double walled cylinder of cellophane tubing and a number of these are mounted between the rims of two wheels attached to a common axis. A large fan at one end circulates the air through the centers of the cylinders while the wheel is rotated (cf. fig. 43). A light dry powder is obtained which can be dissolved readily in sterile distilled water or if desired the plasma can be reconstituted by immersing the sealed cellophane tubing containing the desiccated plasma in ordinary tap water.

The following technic used by Hill for preparing dried plasma is presented because this seems to be a model in efficiency both with regard to quality and quantity of the product.

The donor bloods are collected in individual vacuum bottles (cf. page 142) and stored in the refrigerator. Twice a week blood not used for transfusion is removed from the bank, pooled while still cold in a large stainless metal reservoir and immediately passed through two DeLaval serum separators. (Blood more than 5 days old is not used in order to minimize the possibility of bacterial growth.) The first separator removes the red cells while the second unit acts as a clarifier by removing the fat droplets and residual red cell fragments present in the plasma. A yield of 50.3 per cent of true plasma is said to be obtained by this method. According to Hill the smallest motor-driven separator of the DeLaval type used by him separated as much as 2½ liters in 35 minutes. On the other hand Strumia et al. using a large international centrifuge (cf. page 155) could separate only 14 to 16 liters of blood in an 8 hour day.

After clarification by the second DeLaval separator the plasma collects in a stainless steel container from which it is drawn through a sterile rubber tube into the reservoir of a Seitz filter. If the system is properly balanced the plasma should filter at the same rate as it is separated, making possible a continuous process. This is accomplished by using a filter of proper size; filter discs up to 2 feet in diameter being available.⁷⁷ Plasma that cannot be filtered at once is frozen and kept in that state until it can. The filtered plasma is desiccated by the adtevac process (cf. page

⁷⁵ *Ann Surg* 112: 502 (1940).

⁷⁶ *Jour Amer Med Assoc* 115: 1989 (1940). Cf. *Thalhimer Proc Soc Exp Biol and Med* 37: 639 (1938).

⁷⁷ To facilitate the filtration of the plasma Muirhead and Hill [*Ann Int Med* 16: 286 (1942)] have advised the addition of filter cel to the plasma before filtration. This substance is a form of diatomaceous earth manufactured by Johns-Manville Sales Corp. N Y C. More recently Hill (meeting of the American Serum Association, June 8, 1942) has reported that he no longer filters the plasma at all since he has found that the second centrifugation in the DeLaval separator serves to remove not only platelets and particles of cells but even bacteria so that the plasma obtained in this way is sterile.

156) using large pyrex ampoules of capacities ranging up to 100 c c. Material dried for 48 hours may contain as little residual water as 0.03 per cent. The dried plasma can be obtained in a shorter time by using smaller ampoules but Hill considers larger loads preferable in order to cut down the labor required.

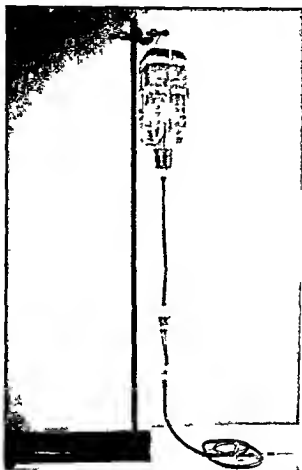


FIG. 44 EQUIPMENT SET UP FOR ADMINISTRATION OF PLASMA TO RECIPIENT

Note stainless-steel filter and tube in reservoir which is used for air vent

(From Muecher *Hospital Progress Bulletin* 163
October 1941)

While the final container may be practically filled with the granular dry plasma because of the porosity of the dry plasma an equal volume of water can be added without difficulty to prepare liquid plasma when needed. The ampoule containing the desiccated plasma is kept sealed under vacuum. Dried plasma which is to be used for the preparation of concentrated plasma is transferred to smaller vials with the aid of a sterile metal dispenser and this container is then sealed under vacuum.

The rapid solubility of the end product is an indication of its proper preparation and advantageous as a time-saver

The transfusion of the plasma to the patient is carried out in the same manner as the transfusion of citrated blood (cf page 98) It is important, as has already been mentioned, that plasma stored in the liquid state be filtered through several layers of gauze, or better, with the aid of a stainless steel filter drip, such as is used for transfusing stored blood (cf fig 44) Filtration is not essential when stored serum is transfused Since plasma and serum are less viscous than blood, a smaller needle (e g, 20 gauge) can be used for puncturing the patient's vein than is necessary for transfusing blood When the plasma has been stored in the dried state, sterile distilled water must first be added to redissolve it Plasma that has been desiccated in the frozen state (lyophilized plasma) can be purchased on the open market together with sterile distilled water and needles and tubing for the transfusion of the reconstituted plasma Most workers recommend that the plasma be allowed to run into the patient's vein at a rate of about 10 c c per minute However, the rate can be varied according to circumstances, it being increased in emergencies and decreased where the patient's cardiac status requires it Hypertonic plasma can be prepared by dissolving the desiccated plasma in about one fourth or one half the original volume of water Such plasma is best transfused by the syringe or syringe valve method

While the pH of fresh citrated plasma varies between 7.4 and 7.7, dried plasma restored with distilled water is highly alkaline,⁷⁸ its pH usually ranging from 8.2 to 9.3.⁷⁹ The alkalinity of the regenerated plasma is probably the cause of the pain in the vein experienced by patients, particularly when such plasma is transfused rapidly Moreover, Strumia⁷⁹ reports that while the preservation of prothrombin is good up to a pH of 7.7 it is poor when the pH is 8.2 or higher Strumia found that the excessive alkalinity could be avoided and a good prothrombin content maintained by dissolving the dried plasma in 0.1 per cent citric acid instead of the customary distilled water

Purified Plasma Proteins As Schiff and Boyd⁸⁰ have pointed out a purified preparation of any of the proteins in human plasma could theoretically be used as a blood substitute As a matter of fact purified serum albumin has already been used in this way Albumin possesses the advantages of stability and a relatively low molecular weight which latter is responsible for a greater osmotic effect per gram of dried material Schiff and Boyd quote Newbouser and Kendrick⁸¹ who

⁷⁸ Scudder Drew Tuthill and Smith *Bull N Y Acad Med* 17 373 (1941)

⁷⁹ Strumia *Jour Amer Med Assoc* 119 710 (1942)

⁸⁰ Schiff and Boyd *Blood Grouping Technic* page 112 Interscience Publishers New York (1942)

⁸¹ *U S Naval Med Bull* 40 1 (1942)

state Human albumin prepared by Cohn⁸² has been given a limited clinical trial and the results thus far are encouraging Albumin has been administered safely in concentrations up to 30 per cent without untoward reactions and it can be stored in the liquid state without refrigeration Experimental and clinical trials have proved it to be effective in restoring depleted blood volume resulting from shock and hemorrhage Since 100 c.c. of 30 per cent human albumin will provide the same colloidal osmotic pressure when injected intravenously as 1000 c.c. of whole blood or 500 c.c. of plasma it is therefore possible to package albumin in a small compact unit ready for immediate use The fact that albumin can be packaged in small containers is of tremendous importance to our military forces

ADDENDUM

While this book was in press an excellent paper by Mollison and Young⁸³ appeared in which are described experiments to determine the *in vivo* survival in the human subject of transfused erythrocytes after storage in various preservative solutions As these authors point out the commonly accepted *in vitro* tests are fallacious indicators of the state of preservation of blood for example while dextrin and sucrose delay the onset of hemolysis *in vitro* they have very little effect in prolonging the *in vivo* survival of the blood The experiments do demonstrate conclusively however, the preservative action of glucose so that while blood stored in citrate alone should not be transfused after periods longer than 5 to 7 days blood stored in suitable glucose-citrate mixtures is satisfactory for periods up to 14 to 21 days Among the methods to be recommended is that advocated by the Medical Research Council of Great Britain namely to mix 100 c.c. of 3 per cent sodium citrate 10 c.c. of 30 per cent glucose solution and 430 c.c. of blood

SPECIAL BIBLIOGRAPHY

The following monographs are recommended to those readers desiring further detailed information concerning the transfusion of stored blood and blood substitutes

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MUDD S. and THALHIMER W. (editors) *Blood Substitutes and Blood Transfusion* Charles C. Thomas Springfield Ill. (1942)

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A Technical Manual on the Preservation and Transfusion of Whole Blood U. S. Office of Civilian Defense Washington D. C.

⁸² *Chem. Rev.* 2: 395 (1941)

⁸³ *Quart. Jour. Exp. Physiol.* 31: 359 (1942)

CHAPTER X

SOME FUNDAMENTAL PRINCIPLES OF GENETICS AND BIOMETRICS

AS AN introduction to the discussion of the heredity of the blood groups, it will be convenient to recapitulate some of the principles of genetics. Two of the fundamental laws were laid down by Gregor Mendel in 1865, after eight years of painstaking and thorough research. The manner of operation of Mendel's laws is best illustrated by considering actual experiments.

Law of Segregation Mendel crossed a tall variety of edible pea with a short variety and obtained in the first generation (F_1) hybrid plants, all of which were tall. When the hybrid tall plants were allowed to self fertilize, three quarters of the offspring were tall and one quarter were short. Mendel explained these observations by postulating that tall plants had something (now known as a gene) in the germ cells that in the offspring determines the character "tall," and similarly, the short plants had something in the germ cells determining the character "short."

Furthermore, all the cells other than the germ cells contain a double set of genes: one derived from the maternal and the other from the paternal parent. Thus, the hybrids receiving one gene from each parent will then possess a gene (T) for tall and a gene (t) for short. Since the hybrids are all tall, tall is said to be "dominant" over short, or short to be "recessive" to tall. In the second (F_2) generation, ova and pollen bearing the genes, T and t , will be produced in equal number. On self fertilization, one fourth of the plants resulting will have the constitution (genotype TT , one half Tt , and one quarter tt , as shown in figure 45, so that three quarters of these plants will be tall and one quarter short.

The essential features of the law of segregation, therefore, are the following: A factor (or gene) carried by the gametes of one parent and a contrasting factor carried by the gametes of the other parent come together and coexist for one generation in the cells of the hybrid offspring *without blending or losing their identity*. When such a hybrid individual produces its own gametes, the two factors are again separated, or *segregated* from one another, each of the gametes being pure, containing either one factor or the other, but never both.

Pairs of genes like T and t (governing contrasting characteristics) are termed "allelic" genes. They have been shown to be located opposite one another on a pair of chromosomes.

Individuals of genotypes TT and tt if self fertilized will breed true,

are therefore known as "pure" types. They are also called "homozygous," since they are produced by two germ cells bearing the same gene. Individuals of genotype Tt are said to be "heterozygous" and will not breed true.

Law of Independent Assortment Mendel crossed peas whose seeds were yellow and round with others whose seeds were green and wrinkled. Previous experiments had shown that yellow and green are contrasting characters, determined by a pair of allelic genes, G and g respectively, where G is dominant over g, and that these characters gave a 3 to 1 ratio in the second generation. The same had been found to be true of the characters round and wrinkled, depending on the allelic pair of genes W and w, respectively.

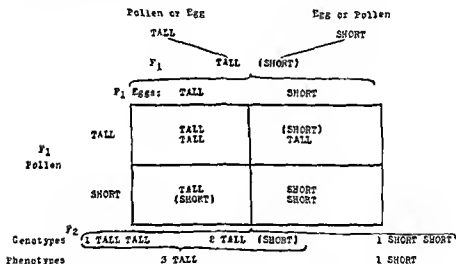


FIG. 45. DIAGRAM ILLUSTRATING THE LAW OF SEGREGATION (After Morgan)

The offspring of the first (F₁) generation were all yellow and round, but when they were self-fertilized, four kinds of individuals resulted: yellow round, yellow wrinkled, green round, and green wrinkled in the ratio of 9 : 3 : 3 : 1. Mendel pointed out that the results obtained could be explained by assuming that the genes for yellow and green segregated independently of the genes for round and wrinkled. The original yellow and round plants, which were "pure" since they bred true, were of genotype GGWW, whereas the original green and wrinkled plants were of genotype ggww. The former produced ovules and pollen grains GW, and the latter produced ovules and pollen grains gw, so that the hybrid plants were of genotype GgWw. Because of the dominance of G over g, and W over w, all the hybrid plants were yellow and round.

According to Mendel's assumption that the genes G and g segregated independently of W and w , the hybrid must have produced four kinds of germ cells, GW , Gw , gW , and gw . Assuming that fertilization of the four kinds of ova by the four kinds of pollen grains occurs at random, sixteen combinations are possible. Taking into account the dominance of yellow over green and of round over wrinkled, we find that these

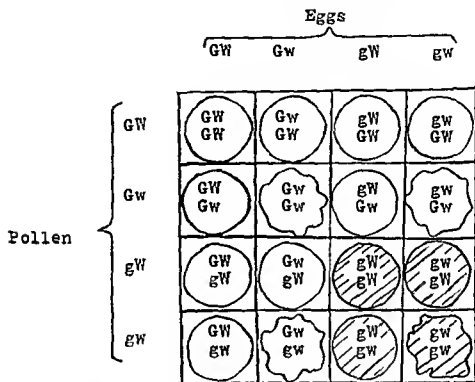


FIG. 46. DIAGRAM ILLUSTRATING THE LAW OF INDEPENDENT ASSORTMENT

Showing the sixteen F_2 recombinations (from yellow round and green wrinkled peas) that result when the four kinds of eggs and the four kinds of pollen grains of the F_1 hybrid come together

(After Morgan)

sixteen combinations fall into four phenotypes in the ratio of 9 : 3 : 3 : 1 (cf fig 46)

Linkage Mendel's law of independent assortment has its basis in that the independent factors are located in different (pairs of) chromosomes (cf fig 47). One member of each pair of chromosomes is present in each germ cell, and since only chance determines which member of the pair enters a particular germ cell, characters determined by genes located in different chromosomes are inherited independently of one another. On the other hand, when two genes are located in the same chromosome, the

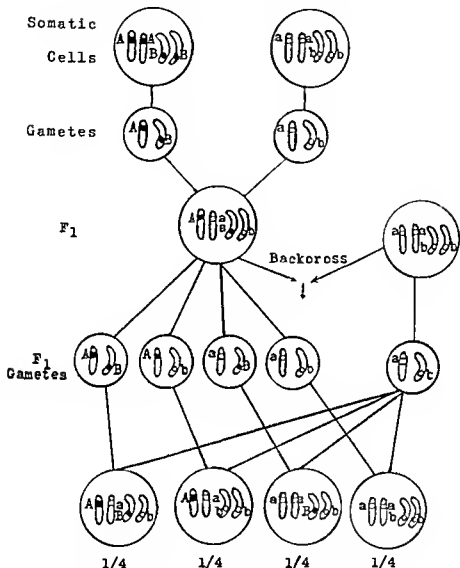


FIG 47 PHYSICAL BASIS OF THE LAW OF INDEPENDENT ASSORTMENT

Since the two pairs of genes *Aa* and *Bb* are located in different chromosomes they segregate independently of one another. The hybrid of genotype *AaBb* therefore produces equal numbers of germ cells of types *AB*, *Ab*, *aB* and *ab*. On back crossing the hybrid with a double recessive individual individuals of genotypes *AaBb*, *Aabb*, *aaBb* and *aabb* are produced in equal numbers.

law of independent assortment will not hold (cf fig 48). Bateson and Punnett found that when a sweet pea having purple flowers and long pollen grains was crossed with one having red flowers and round pollen

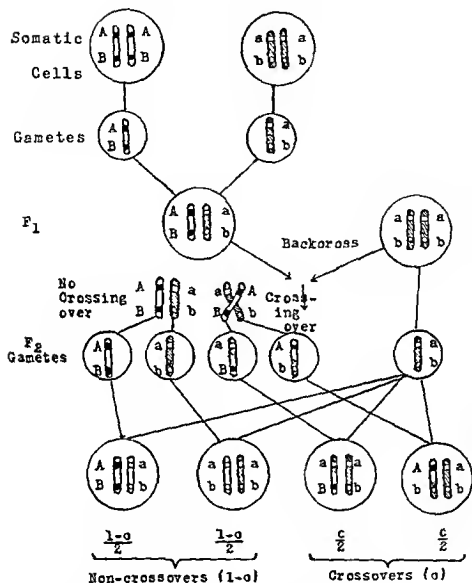


FIG. 48 PHYSICAL BASIS OF LINKAGE

Since the two pairs of genes *A* *a* and *B* *b* are located in the same pair of chromosomes the hybrid is of genotype (*AB*) (*ab*) having received chromosome (*AB*) from one parent and chromosome (*ab*) from the other. Most of the germ cells produced by the hybrid will therefore be (*AB*) and (*ab*) gametes (*aB*) and (*Ab*) also are formed however because of crossing-over. By back crossing the hybrid with a double recessive individual the relative frequency of the crossovers and the non crossovers is determined. If the original parental types had been (*Ab*) (*Ab*) and (*aB*) (*aB*) respectively the genotype of the hybrid would have been (*Ab*) (*aB*) and crossing-over would occur in the opposite direction.

grains, all the offspring had purple flowers and long pollen grains. When the hybrids were self fertilized four different types of offspring were obtained, but not in the ratio 9 3 3 1, there was a large excess of plants with the original combinations, namely, purple flowers and long pollen grains, and red flowers and round pollen grains. This phenomenon is called linkage because genes located in the same chromosome tend to remain together.

The fact that in the example just cited the parental properties namely, purple flowers with long pollen grains, and red flowers with round pollen grains, are not completely linked constitutes a phenomenon known as "crossing over." The cytological counterpart of this phenomenon is the frequent occurrence of a certain amount of interchange of chromatin material in the process of formation of the germ cells. In a general way, the frequency of crossing over is proportional to the distance between the linked genes since the farther away they are the more frequently will they be separated by interchange of chromatin material.

From the relationship between frequency of crossing over and the distance between linked genes, it is possible to plot the genes on a chromosome map. Such a chart has been constructed for the *Drosophila melanogaster* (by Morgan and his co workers).

Sex Linkage. In determining sex, one particular pair of chromosomes plays the main rôle. Thus in man who possesses twenty four pairs of chromosomes it has been found that in twenty three pairs the two members of each pair are alike, whereas in the twenty fourth pair there are two dissimilar chromosomes: a large chromosome (X) and a very small chromosome (Y). Individuals possessing two X chromosomes are females (XX) and individuals with one X and one Y chromosome are males (XY). The Y chromosome is supposed to contain only few genes, whereas the X chromosome contains many genes like the other chromosomes. Genes located in the X or Y chromosome are said to be sex linked.

As an illustration a simple case of sex linkage in man namely hemophilia may be cited, and this is also pertinent because attempts have been made to demonstrate linkage between blood groups and hemophilia (cf. footnote 66, page 374). The heredity of hemophilia has been shown to depend upon a recessive gene *h*, the allelomorph of which is a normal dominant gene. This pair of genes is present in the X but not in the Y chromosome. If X represents an X-chromosome with the gene *h* and X a normal X chromosome, five possibilities may occur: XX hemophiliac female, XX, normal female, but carrying gene *h*, XX normal female, XY hemophiliac male, and XY, normal male.

The mode of heredity of hemophilia is illustrated in figure 49. If a

hemophiliac male is wedded to a normal female, all the daughters will be carriers. If the carrier daughters are wedded to normal men, half of the sons will be hemophiliac and half of the daughters will be carriers. Theoretically, hemophiliac females can only result from matings of hemophiliac males with carrier females. In fact, no completely proved case of hemophilia has ever been observed in a woman, and this has

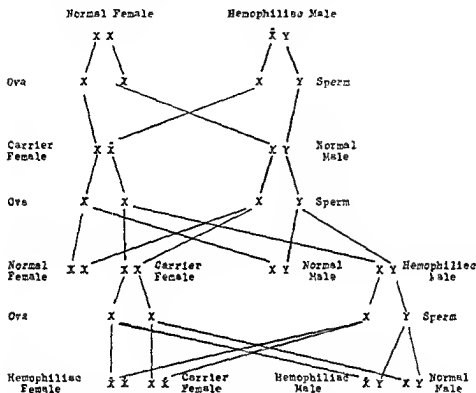


FIG. 49 HEREDITY OF A SEX LINKED CHARACTER HEMOPHILIA

been explained by assuming that the genotype $\bar{X}\bar{X}$ is a so called 'lethal' type, so that such individuals die *in utero*.

SOME BIOMETRICAL CONCEPTS

Probable Error Mendel's laws of heredity have been amply tested and confirmed by hundreds of thousands of observations. In these investigations, as in other biological studies employing statistical considerations, the "probable error" of the observed frequencies must be taken into account. For that reason, a brief resume of the meaning of this term will be given here.

For an understanding of the question an elementary knowledge of the theory of probabilities is necessary. The following two theorems will be found useful.

1. If an event can occur in *a* ways and fail in *b* ways and all these ways are

equally likely, then the chance of its happening is $\frac{a}{a+b}$ and that of its failing is $\frac{b}{a+b}$

For it is obvious that the chance of its happening is to the chance of its failing as a is to b . Then if the chance of its happening is represented by ka where k is a constant still to be determined the chance of its failing is kb . Now the event is certain either to happen or to fail so that the sum of the chances of happening and failing must be certainty which may be designated by unity—

$$\text{Therefore } ka + kb = 1$$

$$\text{and } k = \frac{1}{a+b}$$

Therefore the chance of the event's happening is $\frac{a}{a+b}$ and the chance that the event will not happen is $\frac{b}{a+b}$

For example if a bag contains 4 red and 3 black balls the chances of drawing a black ball at random are $\frac{3}{3+4} = 3/7$. As a corollary to the above theorem if p is

the chance that a given event will occur then the chance that it will fail to occur is $1 - p$

II If the chance that one event happens is p and the chance that another *independent* event happens is p then the chance that both events occur is pp . For if the first event can happen in a ways and fail in b ways all of these being equally likely and if the second event can happen a' ways and fail in b' ways all being equally likely then

$$p = \frac{a}{a+b} \text{ and } p = \frac{a'}{a'+b'}$$

The $a+b$ cases can be associated with the $a'+b'$ cases in $(a+b) \times (a'+b')$ ways all equally likely to occur. In aa' of these both events will happen so that the chance that both will happen together is

$$\frac{aa'}{(a+b)(a'+b')} \text{ or } pp$$

As an illustration of this theorem let us suppose that a bag contains three red and five black balls then the chance of drawing a red ball at random from this bag is $3/8$. Similarly the chances of drawing a red ball at random from a second bag containing one red and two black balls are $1/3$. If one ball is drawn from each bag therefore the chance that they will both be red is $(3/8) (1/3)$ or $1/8$.

The laws just derived also apply to statistical considerations. Thus if 30 per cent of all the people in a population have brown hair and 25 per cent have blue eyes then if the color of the eyes is independent of the color of the hair we should expect to find $(.30) (.25) = 0.075$ or 7.5 per cent of people with brown hair and blue eyes.

As an application of these theorems in probabilities and of the mean

ing of probable error, let us consider the example of tossing a penny. Either a head or a tail will result from each trial, and assuming that either event is equally likely to occur, the chance that a head appears is

$\frac{1}{1+1}$ or $\frac{1}{2}$, and obviously the chance for a tail is the same. We should therefore expect that if a number of such trials were made, half would be heads and half tails.

Suppose, for example that the penny is tossed 100 times. In one experiment there may be 46 heads, 57 in another, etc. and only exceptionally will there be exactly 50 heads. If a large number of experiments are made, and the frequencies are plotted with which no heads out of 100, one, two, up to hundred heads are tossed, a so-called

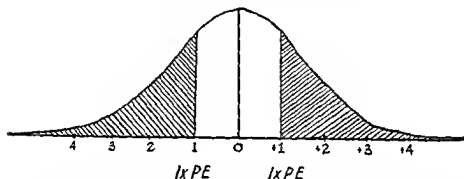


FIG. 50 THE PROBABILITY CURVE

The area of the curve inside (blank) is equal to the area outside (cross hatched). The unit of distance used in the graph is therefore equal to the probable error. If the theoretically expected frequency is at point 0, the chances are equal that an observed frequency fall in the blank or shaded areas.

"distribution curve" is obtained with the mean and the maximum at 50 heads out of a hundred. As the number of experiments increases the distribution curve approaches more and more closely the shape of the "normal curve" (cf. fig. 50).

If on either side of the maximum, and at equal distances from it, two perpendiculars are dropped, so that the area included between the two perpendiculars, the curve and the abscissa is equal to the sum of the two areas outside these perpendiculars, the distance from either perpendicular to the center of the curve is defined as the probable error.

The most frequent application of the probable error concept is for the determination of the statistical reliability of observed frequencies. Thus, suppose one desires to determine the frequency of occurrence of blue eyes in a certain population. A random sample of this population consisting of two hundred individuals is examined and 78 in

dividuals are found to have blue eyes. The frequency of blue eyes in this sample is therefore 0.39 or 39 per cent. The probable deviation of this observed frequency from the actual frequency in the entire population is given by its probable error. If p represents the observed frequency, and N the number of individuals examined, it can be shown that¹

$$P.E._p \text{ (Probable error of } p) = 0.6745 \sqrt{\frac{p(1-p)}{N}}$$

In the present case $P.E._p = 0.6745 \sqrt{\frac{(0.39)(0.61)}{200}} = 0.233$ or 2.33 per cent.

The observed frequency should therefore be expressed as follows 39 ± 2.33 .

If the probable error of an observed frequency is known, the chances that the *true value* lies within any given multiple of $P.E.$ and against its lying outside the range are

- $\pm P.E.$ the chances are even
- $\pm 2 P.E.$ the chances are 4.6 to 1
- $\pm 3 P.E.$ the chances are 21 to 1
- $\pm 4 P.E.$ the chances are 142 to 1
- $\pm 5 P.E.$ the chances are 1310 to 1
- $\pm 6 P.E.$ the chances are 19,200 to 1

Usually, a deviation greater than three times the probable error is considered significant.

European writers use σ or the standard error, as the measure of error, thus simplifying formulae by eliminating the constant 0.6745. The relation between the $P.E.$ and the standard error is given by

$$P.E. = 0.6745 \sigma \text{ so that } \sigma = \sqrt{\frac{p(1-p)}{N}}$$

It is apparent that the larger the number of individuals examined the smaller will be the $P.E.$ and the more reliable will be the observed frequency.

If the calculation is made in terms of absolute values instead of frequencies, in the example just cited the probable deviation would be 2.33 per cent of 200 individuals or 4.66 individuals. In other words, the $P.E.$ in terms of absolute value is equal to N times the $P.E.$ in terms of frequency (per cent). Based therefore on the original observation on 200 individuals, we would expect that in future observations on an

¹ For the derivation of this formula see the books by Kelley and Yule cited at the end of this chapter.

equal number of individuals the number of individuals with blue eyes would fall within the limits 78 ± 4.66 in half of the cases. The chances of finding a deviation of 3×4.66 , or approximately 14 (i.e. less than 64 or more than 92 individuals), would be only 1 in 22, consequently rather small.

Calculation of the probable error may be applied to test the accuracy of theories. Let us use it for example for our penny tossing experiment. Suppose in one experiment of 100 tosses, 47 heads are obtained. Then

$$p = 0.47, N = 100, \text{ and } P.E. = 0.6745 \times \sqrt{\frac{(47)(53)}{100}} = 0.336$$

The theoretically expected frequency is 0.50 (50 heads in 100 tosses). The difference between the observed frequency (0.47) and the theoretical frequency (0.50) is 0.03 so that $\frac{\text{Dev}}{P.E.} = \frac{0.03}{0.336} = 0.09$. Since the

chances for a deviation up to the probable error are even, the experiment cited would satisfy the expectations.

The application of the theory of probable error to the results of experiments in heredity in plants and animals have resulted in complete confirmation of the two laws discovered by Mendel. For example, Johannsen² has summarized the results of counts totalling 173,399 on the heredity of cotyledon color in *Pisum*. The deviation from the expected 3 to 1 ratio was only 0.035 with a probable error of ± 0.028 .

Therefore, $\frac{\text{Deviation}}{P.E.} = 1.25$. Since a deviation of this magnitude could be expected approximately twice in five times, the results of these experiments fully confirm Mendel's first law.

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² Cited after Babcock and Clausen. *Genetics in Relation to Agriculture*. p. 80. McGraw Hill Book Co. New York (1918).

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CHAPTER XI

HEREDITY OF THE BLOOD GROUPS

INTRODUCTION

WHILE A LARGE body of information has been accumulated concerning the genetics of many plants and animals comparatively little is known concerning heredity in man. The main reason for this is that direct experimentation is obviously impossible in studying human inheritance and usually data can only be gathered by the less exact method of analyzing pedigrees and family histories. Nevertheless a number of abnormal characters in man have been shown to be definitely inherited, such as brachydactyly "clawhand" polydactyly, white forelock, hemophilia, etc. Because in many cases the data are based only on the memory of a few individuals in the family they may be inaccurate, and since the number of families studied is greatly limited on account of the rareness of most of these anomalies, not in every case do the Mendelian ratios obtain accurately. These results do not in any way invalidate Mendel's laws as applied to human beings, however, as there is no intrinsic difference in this respect between man and other animals, the difference obtained being caused only by our inability to make direct breeding experiments with large progenies and a large number of generations.

The blood groups afford an excellent opportunity for heredity studies since every family is a source of data on account of the universal distribution of the blood properties. The first report on the heredity of the blood groups was made by Ottenberg and Epstein¹ in 1908. In 1910 as a result of a study of the blood groups in 72 families with 102 children, von Dungern and Hirsfeld² demonstrated that the agglutinogens A and B are each inherited as simple Mendelian dominants. This conclusion has been substantiated by the work of a great number of investigators and is now universally accepted.

However, three main theories have been proposed concerning the exact mechanism of heredity of the agglutinogens A and B (cf. fig. 51). Von Dungern and Hirsfeld thought that A and B were inherited independently of each other, and that their inheritance depended upon two independent pairs of allelic genes. In 1924, Bernstein³ showed that

¹ Epstein and Ottenberg *Trans. New York Pathol. Soc.* 8: 187 (1908).

² v. Dungern and Hirsfeld *Ztschr. f. Immunitäts* 6: 284 (1910).

³ *Klin. Woch.* 3: 1495 (1924). *Ztschr. f. indukt. Abstamm. u. Vererbungslehre* 37: 237 (1925).

the available statistical data did not conform to that theory, and postulated that the inheritance of the agglutinogens A and B depended upon three allelic genes *A*, *B* and *O*. As a sort of compromise between the two theories, Kiriwara and Haku⁴ and, later, Bauer⁵ suggested a theory of linkage for the heredity of the blood groups.

At present all investigators agree that Bernstein's theory is correct,

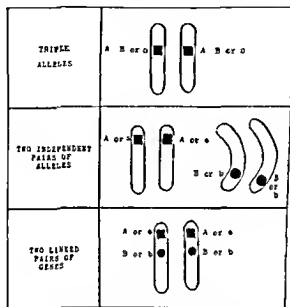


FIG. 51. DIAGRAM ILLUSTRATING THE THREE PROPOSED THEORIES OF THE HEREDITY OF THE BLOOD GROUPS

as has been conceded by Hirszfeld himself. Nevertheless for the presentation of the results of studies on the heredity of the blood groups it is convenient first to discuss the original theory of von Dungern and Hirszfeld.

THE HYPOTHESIS OF TWO INDEPENDENT PAIRS OF GENES

According to the theory of von Dungern and Hirszfeld the heredity of the agglutinin A depends upon the a pair of allelic genes *A* and *a*, where *A* is dominant over *a*, and similarly, the heredity of the agglutinin B depends upon an independent pair of allelic genes *B* and *b*. The

⁴ *Nagoya Jour Med Sci* 2: 75 (1927)

⁵ *Klin Woch* 7: 1583 (1928)

⁶ *Klin Woch* 10: 1910 (1931)

possible genotypes of individuals belonging to each of the four groups in accordance with this theory, are given in table 13

If the heredity of the agglutinin A is considered alone, two phenotypes only are possible individuals possessing agglutinin A (who could be designated as A +), and individuals lacking agglutinin A

TABLE 13
THEORY OF VON DUNGERN AND HIRSZFELD

Phenotype	Genotype		
	Homozygous	Monohybrid	Dihybrid
O	<i>aabb</i>		
A	<i>AAbb</i>	<i>Aabb</i>	
B	<i>aaBB</i>	<i>aaBb</i>	
AB	<i>AABB</i>	<i>AaBB</i> <i>AABb</i>	<i>AaBb</i>

(who could be designated as A—) Three genotypes are possible, namely, *AA*, *Aa* and *aa*. Individuals of the first two genotypes will possess agglutinin A, and individuals of genotype *aa* will lack agglu

TABLE 14
HEREDITY OF THE AGGLUTININ A

Genotypes of Parents	Phenotypes of Parents	Per Cent Children of Genotypes		
		<i>AA</i>	<i>Aa</i>	<i>aa</i>
1 <i>AA</i> × <i>AA</i>	<i>A</i> + × <i>A</i> +	100	0	0
2 <i>A</i> + × <i>Aa</i>		50	50	0
3 <i>Aa</i> × <i>Aa</i>		25	50	25
4 <i>AA</i> × <i>aa</i>	<i>A</i> + × <i>A</i> —	0	100	0
5 <i>Aa</i> × <i>aa</i>		0	50	50
6 <i>aa</i> × <i>aa</i>	<i>A</i> — × <i>A</i> —	0	0	100
		Phenotype <i>A</i> +		Phenotype <i>A</i> —

tinogen A. Six crosses of these three genotypes are possible as shown in table 14. Since no reliable serological method has been devised for differentiating individuals homozygous or heterozygous with regard to agglutinin A, the results obtained are summarized in terms of the phenotypes as shown in that table. It is immediately apparent, therefore, that the following law must hold. If neither parent possesses ag

glutininogen A, none of the children can possess that agglutininogen. By similar reasoning, the same law must hold for the agglutininogen B.

If one considers the agglutinogens A and B together, under the assumption made by von Dungern and Hirsfeld that they are transmitted independently of each other, then the results are as given in table 18.

Since 1910, more than 50 independent workers have made studies on the heredity of the Landsteiner blood groups. In a summary published by Lattes in 1934 there was a total of 12,843 families with 27,418 children. A small percentage of apparent exceptions to the law of von Dungern and Hirsfeld was found. However, the great majority of these apparent exceptions occurred in the earlier studies and may properly be attributed, in large measure, to faulty technic. Therefore in table 15 we have prepared a summary of only those studies on the heredity of the blood groups which were published after 1924 (and for the period 1924-1932 only those which include more than 250 children). In this combined series of 10,628 families with 24,343 children there were only 45 apparent exceptions to the theory of von Dungern and Hirsfeld, and in not one of these cases could the possibility of illegitimacy be excluded. Consequently, it can be safely stated and the opinion on this point is unanimous, that *there is not a single completely proven exception to the law that the agglutinogens A and B do not appear in the blood of a child unless present in the blood of one or both parents*.

Statistical Considerations. As Bernstein was the first to point out, when the theory of von Dungern and Hirsfeld is analyzed from the statistical standpoint, it does not conform to the expectancies.

Let us first consider the heredity of the agglutininogen A alone dependent upon the allelic genes A and a .

Let p = frequency of germ cells in the population containing gene A

\bar{p} = frequency of germ cells in the population containing gene a

Then $p + \bar{p} = 1$ (or 100 per cent), by definition.

If selective breeding and mutation are excluded, the relative frequencies of the genes A and a remain constant from generation to generation. (This is based also on the assumption of equal viability of the germ cells bearing the various genes.) On the other hand, the frequencies of the phenotypes of $A+$ and $A-$ only remain constant if the population is homogeneous. For heterogeneous populations it can readily be shown that equilibrium with respect to a single character like the agglutininogen A, dependent upon a single pair of allelic genes, is reached after one generation of random intermarriage.¹

¹Hardy *Science*, 28: 49 (1908). Wright *Genetics* 6: 162 (1921). The general theory of genetic equilibrium in a population has been given by Kemp [Genetics 14: 83 (1929)].

By reasoning analogous to that given on page 168, at equilibrium, the frequencies of the three genotypes are *

TABLE 15

SUMMARY OF PUBLISHED STUDIES ON THE HEREDITY OF THE BLOOD GROUPS
(For the years 1924-1932 only those studies are included in which a total of 250 or more children were examined)

Parental Combination	Number of Families	Number of Children in Each Group*				
		O	A	B	AB	Totals
O × O	1563	3772	(14)	(9)	0	3795
O × A	2903	2707	3749	(10)	(1)	6467
A × A	1385	556	2538	0	(2)	3096
O × B	1456	1418	(7)	1831	(1)	3257
B × B	554	203	(1)	1009	0	1213
A × B	1400	605	957	771	848	3181
O × AB	530	(8)	633	646	(7)	1290
A × AB	455	0	533	247	312	1092
B × AB	323	(2)	183	406	232	823
AB × AB	59	0	28	36	65	129
Totals	10 628	9 271	8 643	4 965	1 464	24 343

* Numbers in parentheses represent contradictions to the heredity theories (v. Dungern and Hirschfeld Bernstein)

This table includes

- 200 families with 695 children by Snyder [*Genetics* 9 465 (1924)]
- 1535 families with 3626 children by Furuhata Ichida and Kishi [*Japan Med World* 7 1 (1927)]
- 179 families with 390 children by Czorsz [*Klin Woch* 34 1586 (1926)]
- 289 families with 816 children by Sievers [*Acta Pathol et Microbiol Scand* 285 (1927)]
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- 496 families with 496 children by Graff and Werkgarter [*Beitr Z gerichtl Mediz* 7 98 (1928)]
- 50 families with 257 children by Morville [*Acta Path et Microbiol Scand* 6 39 (1929)]
- 96 families with 305 children by L. and H. Hirschfeld [*Ztschr f Immunstats* 54 81 (1927)]
- 1000 families with 1024 children by Haeelhorst [*Ztschr f Konstitutionslehre* 16 227 (1931)]

(Continued at foot of next page)

* The random association of two genes one from the mother and the other from the father mathematically presents the same problem as the drawing of 2 balls from 2 different bags as in the example given on page 168

frequency of $AA = p^2$

frequency of $Aa = 2p\bar{p}$

frequency of $aa = \bar{p}^2$

The frequency of genotype AA is p^2 , since it results from the union of two germ cells, each bearing gene A , and each therefore having the

- 166 families with 685 children by Landsteiner and Levine [*Jour Exp Med* 48 731 (1928)]
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- 355 families with 971 children by Ichida [*Jour Immunol* 16 81 (1929)]
- 121 families with 318 children by Oku [*Okayama Igakkwai Zasshi* 42 2693 (1930)]
- 249 families with 256 children by Karwan Taylor [*Jour Path and Bacteriol* 33 313 (1930)]
- 156 families with 695 children by Wiener and Varsberg [*Jour Immunol* 20 371 (1931)]
- 300 families with 300 children by Buchnov [*Ginekol* 10 338 (1931)]
- 68 families with 215 children by Schiff and Sasaki [*Ztschr f Immunstats* 77 129 (1932)]
- 2317 families with 4782 children by Buning [*Alin Bloch* p 202 (1932) *Geneesk Tyds Nederl Ind* 72 332 (1932)]
- 240 families with 325 children by Lattes and Garrasi [*L Individualità del Sangue* p 129 (1934)]
- 216 families with 839 children by Okaya [*Hanzogaku Zasshi* 8 106 (1934)]
- 287 families with 570 children by Clausen [Undersøgelser over de Serologiske Blodtypegenskaber M og N Copenhagen (1934)]
- 100 families with 220 children by Hyman [*Jour Immunol* 29 223 (1935)]
- 361 families with 739 children by Moureau [*Rev de belge des Sci Med* 7 (1935)]
- 56 families with 205 children by Zieve Wiener and Fries [*Imm Eng* 7 163 (1936)]
- 103 families with 313 children by Matta [Publication No 11 of the Faculty of Medicine Egyptian University (1937)]
- 107 families with 248 children by Taylor and Prior [*Imm Eng* 8 343 (1938)]
- 94 families with 234 children by Holford [*Jour Inf Dis* 63 287 (1938)]
- 65 families with 252 children by Okaya [*Hanzogaku Zasshi* 4 583 (1938)]
- 81 families with 193 children by Hursfeld and Kostuch [*Schwartz Ztschr f Allg Path u Bakt* 1 23 (1938)]
- 110 families with 428 children by Dahr [*Ztschr f Immunstats* 97 170 (1939)]
- 98 families with 314 children by Dahr, Weber and Offe [*Ztschr f Rassenphysiol* 11 78 (1940)]
- 60 families with 238 children by Landsteiner and Wiener [*Jour Exp Med* 74 309 (1941)]

frequency p . For the same reason the frequency of aa is \bar{p}^2 . Genotype Aa may arise in two ways either by the union of a sperm carrying gene A and an ovum carrying gene a , or by the union of a sperm carrying gene a and an ovum carrying gene A , so that the frequency of genotype Aa is $2p\bar{p}$. Obviously the frequencies of the three possible genotypes must add up to 100 per cent (or unity). Thus, $AA + Aa + aa = p^2 + 2p\bar{p} + \bar{p}^2 = (p + \bar{p})^2 = 1$. Similarly, if the frequencies of the

TABLE 16
FREQUENCIES OF THE FOUR BLOOD GROUPS
(Theory of von Dungern and Hirschfeld)

Groups*	Genotypes	Frequencies
O	$aabb$	$\bar{p}^2 \bar{q}^2$ (1)
A	$AAbb$ $Aabb$	$\left. \begin{matrix} p^2 \bar{q}^2 \\ 2p\bar{p} \bar{q}^2 \end{matrix} \right\} = (1 - \bar{p}^2) \bar{q}^2$ (2)
B	$aaBB$ $aaBb$	$\left. \begin{matrix} \bar{p}^2 q^2 \\ \bar{p}^2 2q\bar{q} \end{matrix} \right\} = \bar{p}^2 (1 - \bar{q}^2)$ (3)
AB	$AABB$ $AaBB$ $ABbb$ $ABbB$	$\left. \begin{matrix} p^2 q^2 \\ 2p\bar{p} q^2 \\ \bar{p}^2 2q\bar{q} \\ 2p\bar{p} 2q\bar{q} \end{matrix} \right\} = (1 - \bar{p}^2) (1 - \bar{q}^2)$ (4)

* O, A, B and AB represent the frequencies of the respective groups

genes B and b in the germ cells are q and \bar{q} , respectively, so that q and $\bar{q} = 1$, then,

$$BB + Bb + bb = q^2 + 2q\bar{q} + \bar{q}^2 = (q + \bar{q})^2 = 1$$

Let us now consider the four blood groups O, A, B, AB and the simultaneous heredity of the two agglutinogens, A and B. As we have already stated, the frequencies of the genes A and a (p and \bar{p} , respectively) and of the genes B and b (q and \bar{q} , respectively) remain constant, in the absence of selection, mixture of races, and mutations. If the population is homogeneous, the proportions of the four blood groups will remain constant from generation to generation*. Again by applying the second probability theorem given on page 168, the frequencies of the groups can be expressed in terms of the frequencies of the

* On the other hand if the original population is heterogeneous the departure of the distribution of the groups from that at equilibrium will be halved in each successive generation of random intermarriage [Zarnik *Jahrbuch der Universität Zagreb*, p. 214 (1929). Also see Wentworth and Remick *Genetics* 1: 608 (1916).]

genes. Since, for instance, group O can arise from the combination of two genes *a* (each of frequency *p*) and two genes *b* (each of frequency *q*), the frequency of the phenotype O (genotype *aabb*) is the product of the four frequencies or p^2q^2 . In a similar way the other expressions are derived (cf table 16).

According to the theory of von Dungern and Hirsfeld, therefore, the following relationship between the frequencies of the four blood groups must hold, from equations (1)–(4) in table 16

$$\overline{O} \times \overline{AB} = \overline{A} \times \overline{B} \quad (5)$$

As a matter of fact, in the vast majority of the populations that have been studied thus far this relation does not hold (cf table 21). For this reason the theory that the agglutinogens A and B are inherited independently of one another is not tenable.

The theory of linkage mentioned above is open to the same statistical objection as the theory of von Dungern and Hirsfeld. As Bernstein pointed out in his original paper, the only effect of linkage on the distribution of the blood groups would be to delay the attainment of equilibrium. Therefore, the relation $\overline{O} \times \overline{AB} = \overline{A} \times \overline{B}$ should also hold under such an hypothesis.

THE BERNSTEIN THEORY

According to this theory, instead of two pairs of allelic genes, the existence of three allelic genes *A*, *B*, and *O* is postulated. Under Bern

TABLE 17
BERNSTEIN'S THEORY

Phenotype	Genotype	
	Homozygous	Heterozygous
AB		1B
A	A 1	1O
B	BB	BO
O	OO	

stein's theory, therefore, there is only one locus for the genes determining the blood groups in a single pair of chromosomes, at which locus any one of the three genes may be situated (cf fig 51). This type of heredity, in which instead of the usual pair of alleles, several allelic genes exist, is not uncommon and is known as multiple allelism. Since each germ cell contains only one of the three genes, *A*, *B*, or *O*, from combinations of the three possible kinds of sperms with the three

possible kinds of ova, six different genotypes result. Furthermore, according to Bernstein's theory, genes *A* and *B* are both dominant over *O*, so that the genotypes corresponding to each of the four blood groups are as shown in table 17.

The agglutinogens *A* and *B* being transmitted as mendelian dominants, they cannot appear in the blood of a child unless present in the blood of one or both parents. (In this respect, the Bernstein theory and the theory of von Dungern and Hirsfeld agree.) However, if either parent belongs to group *AB*, his or her genotype is *AB*, so that half of the germ cells will contain gene *A* and half gene *B*. Every child will therefore possess at least one *A* or *B* gene and therefore cannot

TABLE 18

COMPARISON OF THE TWO THEORIES OF HEREDITY OF THE BLOOD GROUPS

Groups of Parents	Groups of Children (v. Dungern and Hirsfeld)	Groups of Children (Bernstein)
1 O × O	O	O
2 O × A	O, A	O, A
3 O × B	O, B	O, B
4 A × A	O, A	O, A
5 A × B	O, A, B, AB	O, A, B, AB
6 B × B	O, B	O, B
7 O × AB	O, A, B, AB	A, B
8 A × AB	O, A, B, AB	A, B, AB
9 B × AB	O, A, B, AB	A, B, AB
10 AB × AB	O, A, B, AB	A, B, AB

belong to genotype *OO* (or group *O*). Similarly, if either parent belongs to group *O*, or genotype *OO*, all the children must possess at least one *O* gene, so that children of group *AB* are impossible. According to Bernstein's theory, therefore, a second law of heredity must hold. The combinations, group *AB* parent with group *O* child and group *O* parent with group *AB* child, are impossible.

The heredity of the blood groups according to Bernstein's theory is given in table 18. The expectancies are identical with the expectancies according to theory of von Dungern and Hirsfeld, except for the last four matings, in which one or both parents belong to group *AB*. Inasmuch as according to the older theory children of all four groups could result, naturally no exceptions to that theory could be found in these matings whereas exceptions might be found to the Bernstein theory.

As a matter of fact, a considerable number of "exceptions" to the

TABLE 19
BLOOD GROUPS OF CHILDREN WITH GROUP AB MOTHERS

Authors	Year	Number of Mothers	Children's Groups				
			O	A	B	AB	Total
1 Ohnesorge*	1925	9	1	3	1	4	9
2 Rech and Wohlsch†	1926	7	0	4	2	1	7
3 Lattes, Badino and Juhasz Schaffer‡	1928	13	0	7	6	0	13
4 Juhasz Schaffer§	1928	20	0	11	6	3	20
5 v Khreninger Guggenberger	1928	7	2	2	2	1	7
6 Preger¶	1928	16	0	8	8	5	21
7 Thomsen**	1928	65	0	35	30	13	78
8 Schiff††	1929	102	0	46	33	23	102
9 Ichida‡‡	1929	15	0	15	7	5	27
10 Liedberg§§	1929	10	0	3	6	1	10
11 Wolff	1929	10	0	5	4	1	10
12 Wiener et al ¶¶	1929						
	1942	89	0	51	38	22	111
13 Havelhorst***	1930	57	0	28	17	12	57
14 Buining†††	1932	227	0	168	208	70	446
15 Wolff and Jonsson‡‡‡	1935	28	0	17	7	4	28
Totals		675	3	403	375	165	946
Per cent			0.31	42.60	39.64	17.44	100.00

* *Zentralbl f Gynäk* 49 2884 (1925)

† *Ztschr f Biol* 84 515 (1926)

‡ *Gior di batteriol e immunol* 3 151 (1928)

§ *Schwetz med Woch* 58 1132 (1928)

|| *Ztschr f Geburtsh u Gynäk* 30 104 (1928)

¶ *Ztschr f Immunitäts* 53 192 (1927)

** *Deutsch Ztschr f d ges gerichtl Med* 10 1 (1927)

†† *Loc cit*

‡‡ *Jour Immunol* 16 81 (1929)

§§ *Acta Path et Microbiol Scand* 6 39 (1929)

||| *Acta Med Scand* 71 54 (1929)

¶¶ Wiener, Lederer and Polayes, *Jour Immunol* 18 201 (1930), Wiener unpublished data

*** *Ztschr f Konstitutionslehre* 15 177 (1930)

††† *Klin Woch* 11 202 (1932)

‡‡‡ *Acta Path et Microbiol Scand* 12 131 (1935)

Bernstein theory have been reported, but a comparison of the number of such cases found before and after the theory was expounded reveals a remarkable decrease during the latter period¹⁰ Thus in the O × AB

¹⁰ See Wiener, Lederer and Polayes *Jour Immunol* 18 201 (1930)

families, whereas 26.26 per cent of exceptions were found before 1926, only 3.61 per cent were found after that date. Similarly in $A \times AB$ families there has been a decrease from 3.77 per cent to 0.87 per cent in the number of exceptions, in the $B \times AB$ families the number of exceptions has been dropped from 11.11 per cent to 0.83 per cent and in the $AB \times AB$ families no exceptions were found at any time. This remarkable decrease in the number of exceptions found to Bernstein's

TABLE 20
BLOOD GROUPS OF CHILDREN WITH GROUP O MOTHERS

	Year	Number of Mothers	Children's Groups				
			O	A	B	AB	Total
1 Ohnesorge	1925	108	85	18	4	1	108
2 Rech and Wohlsch	1926	70	39	22	7	2	70
3 Lattes, Badino and Juhasz-Schaffer	1928	195	135	41	19	0	195
4 Juhasz-Schaffer	1928	147	116	23	12	0	151
5 v. Khreninger-Guggenberger	1928	70	52	14	2	2	70
6 Preger	1928	139	108	33	11	0	152
7 Schiff	1929	788	462	255	71	0	788
8 Ichida	1929	26	22	5	9	0	36
9 Liedberg	1929	86	52	30	4	0	86
10 Wolff	1929	61	39	16	8	0	63
11 Wiener et al.	1929						
	1942	702	465	188	95	0	748
12 Havelhorst	1930	492	314	146	34	0	494
13 Buining	1932	1274	1265	370	646	0	2281
14 Wolff and Jonsson	1935	212	125	63	24	0	212
Totals		4370	3279	1224	946	5	5454
Per cent			60.12	22.44	17.35	0.09	100.00

theory can probably be explained in two ways. Firstly, there has been a notable improvement in the technique. Secondly, these cases did not attract the attention of the authors until Bernstein published his theory, and were therefore not retested.

A certain minimum number of exceptions are to be expected on account of illegitimacy, an uncertainty which is not present in animal and plant studies in genetics. Thus, the combinations group AB fathers with group O children, and vice versa may be due to illegitimacy. On the other hand, combinations of group AB mothers with group O children and vice versa cannot be attributed to illegitimacy and constitute

true exception to Bernstein's theory Schiff¹¹ has therefore suggested a study of the blood groups of mothers and children as a test of the theory. In table 19, are presented the results of the studies of fourteen authors, giving the number of children in each blood group with mothers belonging to group AB. In table 20, a similar summary is presented, the mothers here belonging to group O. In the former table, three exceptions (0.31 per cent), and in the latter table, five exceptions (0.09 per cent), are listed. These eight exceptions were found in only three of the fourteen reports, all of which were performed without knowledge of Bernstein's theory, and which together include less than 5 per cent of the total number of cases examined. The reliability of these observations can therefore reasonably be doubted.

More recent studies have revealed an important source of error in blood grouping which should be taken into account in subsequent work in this field. According to Bernstein's theory, genes *A* and *B* are both dominant over gene *O*, but Bernstein believed that the dominance of *A* and *B* was equal. As has already been mentioned (cf. page 35) however, Thomsen has found that the sensitivity of agglutinin *A* is diminished in group AB, indicating some dominance of gene *B* over gene *A*.¹² Furthermore, there are two types of agglutinin *A*, *A*₁ and *A*₂, and agglutinin *A*₂ is less sensitive to agglutination than *A*₁. In subgroup *A*₂*B*, in particular, the sensitivity of agglutinin *A* may be so decreased that a blood grouping based on the examination of the blood cells alone can lead to an erroneous diagnosis. In new born infants because of the incomplete development of the agglutinogens, an *A* blood may be mistaken for group *O* (cf. also page 26).

This source of error can be illustrated by actual cases that have been reported in the literature. As has already been pointed out according to the Bernstein theory, two laws must hold:

1. The agglutinogens *A* and *B* cannot appear in the blood of a child unless present in the blood of one or both parents.
2. The combinations, group *AB* parent with group *O* child and group *O* parent with group *AB* child are impossible.

A case which could be misinterpreted as an exception to the first law is the one reported by Laguna.¹³ In this family, the serological type of the mother's blood seemed to be *B*, *o*, and of the father's blood was *B*, *a*. There were three children whose blood was like that of the mother (*B*, *o*), four typical group *B* and two group *A* children. By far the most

¹¹ *Klin Woch* 7: 1317 (1928) *ibid* 8: 443 (1929)

¹² There are some indications of a similar diminution in the sensitivity of the *B* agglutinin in bloods of subgroup *A*₁*B* (Wiener unpublished observations)

¹³ *Klin Woch* 9: 547 (1930)

probable explanation was that the true phenotype of the mother and three children with similarly reacting blood was AB, o (subgroup A₂B). The situation in this case was clarified by examining the serum as well as the cells. Since in medicolegal cases both cells and serum are routinely examined, there should be no difficulty from this source.

An apparent exception to the second law was the case reported by Worsaae¹⁴. In this case the mother belonged to group AB (subgroup A₂B) and the infant apparently belonged to group O. Several months after birth however, the agglutinin A₂ could be demonstrated in the child's blood. On the other hand in a case reported by Haselhorst and Lauer,¹⁵ where the mother also belonged to subgroup A₂B, the child's group persistently remained group O, although the blood was repeatedly examined over a period of two years. A similar unexplained exception was reported by Kossovitch.¹⁶

Theoretically, exceptions such as the case of Haselhorst and Lauer might result from mutations although evidence for the recent occurrence of such mutations is lacking. Another explanation offered considers the possibility of non disjunction.¹⁷ A decision can only be reached through family studies, in case further exceptions to the theory are encountered.

A theory to explain Bernstein exceptions could be constructed on the hypothetical assumption of four *completely linked* pairs of genes (Ab) (aB) (ab) and (AB) or what amounts to practically the same thing by assuming the existence of four allelic genes of which three genes are the genes A, B and O of Bernstein's theory and the fourth allelic gene (C) acts like the genes A and B together and determines the presence of both agglutinogens A and B. Naturally the fourth allelic gene (C) if it exists at all would have to be extremely rare. This possibility which was first considered by the writer¹⁸ and independently by Edwards and Etherington¹⁹ would imply the existence of four different genotypes in group AB namely AB CO CA and CB. This would call for the existence for example of certain families O×AB (OO×CO) in which half of the children belong to group O and half to group AB none to group A or to group B. Since such families have not been found the existence of a fourth allelic gene seems very improbable.

It has been suggested that any apparent exceptions to Bernstein's theory be analyzed in the following way:

- 1 Complete examination of cells and serum with several sets of reagents
- 2 Examination of specimens taken at different intervals, especially

¹⁴ *Ann. Hoch* 9: 938 (1930)

¹⁵ *Ztschr. Konstitutionslehre* 15: 205 (1930) *ibid* 16: 277 (1931)

¹⁶ *Rev. Anthropol.* 39: 314 (1929)

¹⁷ Levine

¹⁸ Wiener, Lederer and Polayes *Jour. Immunol.* 17: 218 (1930)

¹⁹ *Nature* 136: 297-646 (1935)

in the case of infants whose blood groups may not be fully developed

3 Confirmation by workers with great experience

4 Determination of the subgroup of any group A or group AB bloods in question

5 Complete study of the family

Statistical Consideration The frequencies of the genes A , B , and O postulated by Bernstein's theory are readily determined when the frequencies of the four blood groups are known, provided that the population is homogeneous. For multiple allelic genes, just as in the case of a single pair of allelic genes, equilibrium is reached after a single generation of random intermarriage. If p , q , and r represent the frequencies of the genes A , B , and O , respectively, and the population in question is homogeneous, then the values of p , q , r may be derived as follows (cf. page 179)

<i>Phenotype</i>	<i>Genotype</i>	<i>Frequencies</i>	
\bar{O}	OO	r^2	(6)

\bar{A}	$\begin{cases} AA \\ AO \end{cases}$	$\begin{matrix} p^2 \\ 2pr \end{matrix}$	$p^2 + 2pr$	(7)
-----------	--------------------------------------	--	-------------	-----

\bar{B}	$\begin{cases} BB \\ BO \end{cases}$	$\begin{matrix} q^2 \\ 2qr \end{matrix}$	$q^2 + 2qr$	(8)
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\bar{AB}	AB	$2pq$	(9)
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Furthermore, by definition $p+q+r=1$ (or 100 per cent)

From (6) $r = \sqrt{\bar{O}}$ (10)

By adding (6) and (7), we obtain

$$\bar{O} + \bar{A} = p^2 + 2pr + r^2 = (p+r)^2$$

so that $p+r = \sqrt{\bar{O} + \bar{A}}$

But $p+q+r=1$ or $p+r=1-q$

Therefore, $1-q = \sqrt{\bar{O} + \bar{A}}$

and $q = 1 - \sqrt{\bar{O} + \bar{A}}$ (11)

Similarly, $p = 1 - \sqrt{\bar{O} + \bar{B}}$ (12)

If the Bernstein theory is correct, the values of p , q , and r given by (10), (11), and (12), must satisfy the relation, $p+q+r=1$ so that,

$$(1 - \sqrt{\bar{O} + \bar{B}}) + (1 - \sqrt{\bar{O} + \bar{A}}) + \sqrt{\bar{O}} = 1 \quad (13)$$

In any given population, of course, relation (13) need not hold *exactly*. (This is analogous to the penny-tossing experiment previously mentioned (cf. p. 171), where it was pointed out that exactly 50 heads out of 100 could not reasonably be expected in every experiment.) If the deviation of the calculated sum, $p + q + r$, from 100 per cent be designated as D , so that $D = 1 - (p + q + r)$, one can determine for any given population whether the deviation found is larger than would be expected as a result of chance alone, provided that the probable error or D is known. According to Bernstein,²⁰ the probable error of D can be calculated from the frequencies of the genes, by means of the following formula:

$$P.E.D = \frac{0.6745}{\sqrt{N}} \sqrt{\frac{pq}{2(1-p)(1-q)}}. \quad (14)$$

A large list of populations is available in which the distribution of the blood groups has been determined. Steffan²¹ lists the results of more than six hundred such studies. Inasmuch as these studies were performed by many different authors, some of whom used a questionable technic, there are many apparent discrepancies. If, however, only reports of workers whose results are known to be reliable are considered, they will be found to agree with the expectancies. E.g., in tables 21 and 22, we have listed fourteen different populations, all of which were tested by H. and L. Hirszfeld.²² As has already been pointed out, in not a single case does the relation $\overline{O} \times \overline{AB} = \overline{A} \times \overline{B}$ hold (cf. table 21). On the other hand, as shown in table 22, the relation, $p + q + r = 1$, where p , q , and r have the values given by formulae (10), (11), and (12), holds quite satisfactorily. From a statistical standpoint, therefore, the available data strongly support the Bernstein theory.

By using the so-called Chi Square method of measuring goodness of fit, Strandkov²³ has also demonstrated that the available statistical data support the Bernstein theory.

Naturally, the calculated values of p , q , and r , being based upon observed frequencies will not satisfy equation $p + q + r = 1$ perfectly (see table 22). For the purpose of substitution in formulae involving p , q and r , it is desirable to have a set of values adding up to the unity. Therefore, Bernstein has derived such a set of formulae which at the same time gives the best statistical fit.²⁴

²⁰ *Ztschr f indukt Abstamm u Vererbungslehre* 54: 400 (1930)

²¹ *Handbuch der Blutgruppenkunde*, p 396, J. F. Lehmann (1932)

²² *Lancet* 2: 675 (1919)

²³ *Jour Immunol* 21: 261 (1931). Also see Stevens, *Ann Eug* 8: 362 (1938)

²⁴ *Ztschr f indukt Abstamm u Vererbungslehre* 56: 233 (1930). Cf. Stevens, *loc cit*

TABLE 21

STATISTICAL TEST OF THEORY OF VON DUNGERN AND HIRSZFELD
(Made with data accumulated by L. and H. Hirszfeld*)

Race	Number of People	Frequencies of Groups				O×AB	A×B
		O	A	B	AB		
English	500	0 464	0 434	0 072	0 031	0 0143	0 0312
French	500	0 432	0 426	0 112	0 030	0 0129	0 0477
Italians	500	0 472	0 380	0 110	0 038	0 0179	0 0418
Serbian	500	0 380	0 418	0 156	0 016	0 0175	0 0642
Greeks	500	0 382	0 416	0 162	0 040	0 0153	0 0674
Bulgarians	500	0 390	0 406	0 142	0 062	0 0241	0 0577
Arabs	500	0 432	0 324	0 190	0 050	0 0218	0 0616
Turks (Macedonia)	500	0 368	0 380	0 186	0 066	0 0243	0 0707
Russians	1000	0 407	0 312	0 218	0 063	0 0256	0 0680
Spanish Jews	500	0 388	0 330	0 232	0 050	0 0194	0 0766
Madagascans	400	0 458	0 262	0 237	0 045	0 0261	0 0621
Senegal Negroes	500	0 432	0 224	0 292	0 050	0 0216	0 0654
Annamese	500	0 420	0 224	0 284	0 072	0 0302	0 0636
Hindus	1000	0 313	0 190	0 412	0 085	0 0266	0 0783

* The table that appears in the paper by L. and H. Hirszfeld also includes statistics concerning the Germans and Austrians. These statistics are not included here since they were cited by *memory* from the studies of von Dungern and Hirszfeld in Germany and of Landsteiner in Vienna.

TABLE 22

STATISTICAL TEST OF BERNSTEIN'S THEORY

Race	p	q	r	$p+q+r$	Dev	P E	Dev
							P E
English	0 268	0 052	0 681	1 001	0 001	0 0037	0 3
French	0 262	0 074	0 657	0 993	0 007	0 0037	1 9
Italians	0 237	0 077	0 687	1 001	0 001	0 0034	0 3
Serbian	0 268	0 107	0 516	0 991	0 009	0 0045	2 0
Greeks	0 262	0 107	0 618	0 987	0 013	0 0044	3 0
Bulgarians	0 271	0 108	0 624	1 003	0 003	0 0045	0 7
Arabs	0 209	0 129	0 660	0 998	0 002	0 0042	0 5
Turks	0 256	0 136	0 607	0 999	0 001	0 0049	0 2
Russians	0 210	0 152	0 638	1 000	0	0 0030	0
Spanish Jews	0 213	0 153	0 623	0 989	0 011	0 0047	2 3
Madagascans	0 168	0 154	0 675	0 997	0 003	0 0046	0 7
Senegal Negroes	0 149	0 189	0 657	0 995	0 005	0 0043	1 2
Annamese	0 161	0 198	0 648	1 008	0 008	0 0045	1 8
Hindus	0 149	0 291	0 560	1 000	0	0 0040	0

$$p = \left(1 - \sqrt{\frac{O+B}{O+A+B}}\right) \left(1 + \frac{D}{2}\right) \quad (15)$$

$$q = \left(1 - \sqrt{\frac{O+A}{O+A+B}}\right) \left(1 + \frac{D}{2}\right) \quad (16)$$

$$r = \left(\sqrt{\frac{O}{O+A+B}} + \frac{D}{2}\right) \left(1 + \frac{D}{2}\right) \quad (17)$$

When the frequencies of the genes are known it is possible to determine what the percentages of children belonging to each group should be for each of the ten possible matings. Thus, in mating $A \times O$, the group A parent could belong to genotype AA or genotype AO . The frequency of mating $AA \times OO$ is $2p^2r^2$, since there are two possibilities: AA father and OO mother, or OO father and AA mother, with the frequency of AA equal to p^2 , and the frequency of OO equal to r^2 . All of the children in this case must belong to group A. The mating $AO \times OO$ can occur in $4pr^2$ ways, and half of the children of this mating belong to group A and half to group O. The frequency of group A children in the mating $A \times O$ therefore is $2p^2r^2 + 2pr^2 = 2pr^2(p+r)$, and the frequency of O children in such a mating is $2pr^2$. In a similar manner, the rest of table 23 is derived.

TABLE 23

GROUP FREQUENCIES OF CHILDREN IN EACH MATING IN TERMS OF p , q , AND r
(After Schiff*)

Groups of Parents	Blood Groups of Children			
	O	A	B	AB
$O \times O$	r^2			
$O \times A$	$2pr^2$	$2pr^2(p+r)$		
$O \times B$	$2qr^2$		$2qr^2(q+r)$	
$A \times A$	p^2r^2	$p^2(p+r)(p+3r)$		
$A \times B$	$2pqr^2$	$2pqr(p+r)$	$2pqr(q+r)$	$2pq(p+r)(q+r)$
$B \times B$	q^2r^2		$q^2(q+r)(q+3r)$	
$O \times AB$		$2pqr^2$	$2pqr^2$	
$A \times AB$		$2p^2q(p+2r)$	$2p^2qr$	$2p^2q(p+r)$
$B \times AB$		$2pq^2r$	$2pq^2(q+2r)$	$2pq^2(q+r)$
$AB \times AB$		p^2q^2	p^2q^2	$2p^2q^2$

* *Technik der Blutgruppenuntersuchung*, p. 68, Julius Springer, Berlin (1932)

The expected frequencies of the children listed in table 23 are expressed as percentages of the total number of children from all matings. If it is desired to analyze each sort of mating separately, the formulae should be divided by the frequency of the mating. In mating $A \times AB$, for example, the frequencies are to be divided by $4p^2q(p+2r)$. Except for the mating $O \times O$, where all the children belong to group O, and the matings where at least one parent belongs to AB, the frequencies of the groups among the children are functions of p , q and r . However, these formulae are not applicable to the data given

in table 15, since this is a compilation of the observations of many authors from different parts of the world with different values of p , q and r . It is possible to test Bernstein's theory, however, by determining how closely the observed distribution of the groups in the children fits with the expected values in those cases where these are independent

TABLE 24

STATISTICAL ANALYSIS OF MATINGS IN TABLE 15 HAVING AT LEAST ONE
GROUP AB PARENT

Comparison of distribution of groups among the children with that expected according to Bernstein's theory.

Parental Combination		Number of Children in Group				
		O	A	B	AB	Total
O × AB	Observed { Number Per Cent	—	633 49.5 ± 0.9	646 50.5 ± 0.9	—	1279 100.0
	Expected (Per Cent)	0	50	50	0	
A × AB	Observed { Number Per Cent	0	533 48.8 ± 1.0	247	312	1092
	Expected (Per Cent)	0	50	$\frac{r}{2(p+2r)}$	$\frac{p+r}{2(p+2r)}$	
B × AB	Observed { Number Per Cent	—	183	406 49.4 ± 1.2	232	821
	Expected (Per Cent)	0	$\frac{r}{2(q+2r)}$	50	$\frac{q+r}{2(q+2r)}$	
AB × AB	Observed { Number Per Cent	0	28 21.7 ± 2.4	36 27.8 ± 2.6	65 50.5 ± 2.9	129 100.0
	Expected (Per Cent)	0	25	25	50	

Figures following \pm sign represent probable errors.

of p , q and r . This has been done in table 24, and it will be seen that in every instance the observed frequencies agree closely with Bernstein's theory.

In all published studies on the heredity of the blood groups, little attention has been paid to the mating $A \times B$ since here children of any group can occur under both proposed theories of heredity. Evidently, it was believed that this mating would throw little or no light on the mechanism of heredity. As is shown below, however, an analysis of this mating furnishes an important index of the reliability of the results reported by each investigator. As has already been pointed out, the

most important cause of apparent exceptions to Bernstein's theory is technical errors. After Bernstein's theory was promulgated, the number of such discrepancies diminished markedly, since such cases were then recognized and retested. In the mating $A \times B$, however, no such check on the technic is available, and only by comparing the observed distribution of the groups in a large series of children with the expected values are such technical errors revealed.

The matings $A \times B$ are of four varieties.

Genotypes of Parents	Per Cent Distribution of Groups in Children			
	O	A	B	AB
$AA \times BB$	0	0	0	100
$AA \times BO$	0	50	0	50
$AO \times BB$	0	0	50	50
$AO \times BO$	25	25	25	25

Accordingly, $(AB - O) \geq (A - O) + (B - O)$, or the excess of group AB over group O among the children should be slightly greater than the sum of the excesses of A over O and of B over O. In table 15, however, we find that in the mating $A \times B$, $(A - O) = 352$, $(B - O) =$

TABLE 25

STATISTICAL ANALYSIS OF MATING $A \times B$ IN PUBLISHED STUDIES* ON THE HEREDITY OF THE BLOOD GROUPS

Years of Studies	Investigators	Number of Families	Number of Children				(AB - O)	(A - O) + (B - O)
			O	A	B	AB		
1910-1924	11 reports	92	26	64	62	38	12	72
1916-1927	Japanese authors	87	27	71	54	64	38	69
1926	Bessiedin	47	10	42	42	22	12	64
1925-1927	Furuhata, Ichida and Kishi	257	125	190	140	146	21	80
1925-1927	Snyder, Schiff and Thomsen (pooled data)	49	24	39	37	53	29	28
1925-1927	11 reports	196	50	146	103	82	32	149
1928-1929	13 reports	425	150	295	226	305	55	221
1930-1932	19 reports	198	78	119	91	82	4	54
1932	Buinig	336	225	285	274	338	113	109
1932-1942	13 reports	158	74	118	96	131	57	66

* The data for studies up to the year 1932 were obtained from Lattes (*L'Individualità del Sangue*, Milano, 1934).

166, while $(AB - O) = 243$, so that $(AB - O)$ is equal to less than half the expected value. The cause for this large discrepancy becomes apparent from inspection of table 25. It is seen that the discrepancies occurred in the reports published before 1932, with the exception of the pooled results of Snyder, Schiff and Thomsen, three of the most outstanding workers in the field, which fit the theory despite the fact that their investigations were carried out as early as 1925-27. The pooled data from 13 reports in the most recent period (1932-1941) differ only slightly from the calculated values and Burnings extensive material (1932) gives an excellent fit. It is significant moreover that the deviations noted in the earlier reports are all in the same direction pointing to the occurrence of similar technical errors.²⁵

As for the nature of the errors probably the most important is the incorrect diagnosis of A₂B blood as group B instead of group AB. Accordingly in matings A₂ × B too many B and too few AB children would be recorded. Such errors would tend to lower the value of $(AB - O)$ and at the same time would raise the value of $(A - O) + (B - O)$.

In studies of blood groups in mothers and children it is possible to predict the frequency of each mother-child combination when the distribution of the blood groups in the population is known. Eg. the frequency of the combination group O mother with group O child is derived as follows:

Group O children result when the father belongs to group O (100 per cent) when he belongs to genotype AO (50 per cent) and also when he belongs to genotype BO (50 per cent). The frequencies of the matings are

$$\begin{aligned} \text{mother } OO \times \text{father } OO &= r^2 \\ \text{mother } OO \times \text{father } AO &= 2pr^2 \\ \text{mother } OO \times \text{father } BO &= 2qr^2 \end{aligned}$$

Therefore the combination group O mother with group O child has the frequency

$$r^4 + pr^2 + qr^2 = r^2(p + q + r) = r^2$$

(since $p + q + r = 1$)

The other frequencies given in table 26 were derived in a similar manner. Schiff was the first to suggest this method of testing the Bernstein theory and his data fully support the theory. Similar results have been obtained by other authors.

Results of blood grouping tests performed in cases of disputed paternity can be analyzed from the standpoint of Bernstein's theory. The present writer has shown²⁶ that in a population such as that existing in New York City the chances of proving non-paternity are 18.05 per cent but if the special Bernstein exceptions are not used this is reduced to 14.75 per cent. Hence the increase in the number of exclusions resulting from Bernstein's law should be approximately 1 in 4 or 1 in 5. For the Swedish population studied by Wolff and Jonsson²⁷ the theoretically expected ratio of Bernstein exclusions to dominance exclusions is 2.15 to 12.69 or 1 to 6.

²⁵ Cf. Taylor, Race, Prior and Ikin *Jour. Path. and Bact.* 54: 81 (1942).

²⁶ *Jour. Immunol.* 19: 259 (1930).

²⁷ *Acta Path. et Microbiol. Scand.* 12: 131 (1935).

approximately This agrees satisfactorily with the ratio actually found by these authors in 883 paternity cases (9 Bernstein exclusions and 58 exclusions based on the dominance law)

On the basis of the above calculations it would seem difficult to explain why in the general table of blood group inheritance which appeared in the first edition of this book there were 106 apparent contradictions to Bernstein's law in comparison to only 60 apparent exceptions to the law of von Dungern and Hirschfeld If these apparent exceptions were entirely due to illegitimacy there should have been a much smaller percentage of exceptions to Bernstein's theory In fact for reasons already pointed out (cf page 182) the majority of reported contradictions to Bernstein's theory can be attributed to faulty technic illegitimacy playing only a minor role In the revised table summarizing only the larger and more recent studies on the heredity of the blood groups (cf table 15) there are only 13 apparent exceptions

TABLE 26

FREQUENCIES OF MOTHER CHILD COMBINATIONS IN TERMS OF p q AND r
(After Schiff)

Group of Mother	Children's Groups			
	O	A	B	AB
O	r^2	pr^2	qr^2	O
A	pr^2	$p(p^2 + 3pr + r^2)$	pqr	$pq(p+r)$
B	qr^2	pqr	$q(q^2 + 3qr + r^2)$	$pq(q+r)$
AB	O	$pq(p+r)$	$pq(q+r)$	$pq(p+q)$

to Bernstein's law in comparison to 45 exceptions to the dominance law giving a ratio of 1 to 3.5 for the two sorts of exceptions Considering the relatively small number of cases involved (58 cases) this does not differ significantly from the expected ratio Moreover the agreement is further improved when one considers that technical errors still cannot be excluded entirely

Recently Matta²⁴ attempted an analysis of the reported exceptions to Bernstein's theory along similar lines but arrived at the conclusion that the published data contradict the theory Matta's erroneous conclusions are due to the improper selection for analysis of only a small portion of the published material available The theory which Matta himself proposes is far less plausible and does not conform with actual observations

Serological Considerations Under the theory of von Dungern and Hirschfeld the blood of group O individuals (genotype $aabb$ or 'Not A, Not B') would be characterized merely by the absence from the erythrocytes of A and B agglutinogens Under the Bernstein theory, on the other hand, it could be expected that such individuals (genotype OO) would possess a special 'O' agglutinin in their erythrocytes Hence the discovery of the existence of sera which selectively agglutinate group O blood²⁵ lends additional support to the latter theory (cf page 201)

²⁴ Publication No. 11 Faculty of Medicine of the Egyptian University 1937

²⁵ Schiff *Ann. N. Y. Acad. Sci.* 6: 303 (1927)

Heredity of the Isoagglutinins Thus far we have considered the heredity of the blood groups only with reference to the agglutinogens, the agglutinins have not been taken into account. From the rule that those isoagglutinins are present for which there are no corresponding agglutinogens, the heredity of the isoagglutinins might be said to depend upon the heredity of the isoagglutinogens. But what is the reason for the reciprocal relation between agglutinin and agglutinogen? Two theories have been suggested either of which would account for the facts.

(1) According to Bernstein²⁰ all individuals produce both α and β agglutinins. It has already been pointed out that the isoagglutinins develop later than the isoagglutinogens (cf. page 21). In an individual of group A, first the agglutinogen A will appear. When the agglutinins α and β are now elaborated, the former agglutinin will be absorbed by agglutinogen A. The effectiveness of this mechanism is enhanced by the presence of group specific substances in practically every tissue in the body (cf. Chapter XVII).

(2) To account for a regular occurrence of both isoagglutinins in individuals of any group, Schiff and Adelsberger²¹ have suggested that every individual possesses not only the antigens characteristic of the group but also those foreign to the group in minute quantities—sufficient to stimulate the production of antibodies but not enough to absorb the antibodies once they are formed. This concept would imply a physiological immunization that continues throughout life. Thus far no experimental evidence has been offered in support of the hypothesis.

(3) Furuhashi²² has offered a different explanation for the appearance of the isoagglutinins. According to Furuhashi the blood groups are inherited by means of two pairs of allelic genes, A and a , B and b , where A is dominant over a , and B is dominant over b . And

A determines appearance of agglutinogen A

a determines appearance of agglutinin α

B determines appearance of agglutinogen B

b determines appearance of agglutinin β

According to Furuhashi's theory, these four genes are transmitted in three completely linked pairs,²³ (Ab), (aB), and (ab) which obviously correspond to the genes A , B , and O of Bernstein. Consequently this theory yields the same results with regard to the heredity of the blood groups as the Bernstein theory. The only difference between the two theories is that according to Furuhashi incompatible agglutinins are not

²⁰ *Loc. cit.*

²¹ *Centralbl. f. Bakter. Parasitenk. u. Infektion* 93: 172 (1924).

²² *Japan Med. World* 7: 197 (1927).

²³ Cases of complete linkage such as the one postulated by Furuhashi are ordinarily considered by geneticists as multiple effects of a single gene.

formed, whereas according to Bernstein they are formed but eliminated. The genotypes corresponding to each of the four blood groups according to Furuhashi's theory, are shown in table 27.

Friedenreich²⁴ has recently pointed out that although either of the two hypotheses discussed explains the regular relationship between the isoagglutinogens and isoagglutinins, the former is more general in so far as it can more easily be extended to explain the occurrence of irregular

TABLE 27
HEREDITY OF THE BLOOD GROUPS ACCORDING TO FURUHATA

Phenotype	Genotype	
	Homozygous	Heterozygous
O	(ab) (ab)	
A	(Ab) (Ab)	(Ab) (ab)
B	(aB) (aB)	(aB) (ab)
AB		(Ab) (aB)

agglutinins in the subgroups of group A and group AB (cf. page 204). For this reason, in his opinion, the hypothesis offered by Bernstein seems more likely to be correct.

According to Dupont²⁵ the isoagglutinins are acquired characteristics resulting from oral immunization with animal foods and possibly bacteria containing A and B antigens. But this purely hypothetical idea does not account for the regularity in the occurrence of isoagglutinins also in peoples with entirely different food habits.²⁶ Moreover in favor of the constitutional nature of the isoagglutinins may be mentioned again the regular presence of anti A agglutinins in certain monkey species and of anti B agglutinins in other monkey species (cf. page 30). Finally while the titers of the isoagglutinins of different individuals vary considerably the titer tends to remain constant in the same individual and according to Buhler²⁷ there is a much higher correlation between the isoagglutinin titers in monozygotic twins than in dizygotic twins.

Constancy of the Blood Groups. As we have already pointed out, the phenotype results from the interaction between the germ plasm and the environment. Thus, if one of two plants of identical genotypes is planted on rich soil, and the second is planted on poor soil, the former will grow normally whereas the growth of the second may be stunted.

²⁴ *Ztschr f Immunstats* 71: 1 (1931).

²⁵ *Arch Internat de Méd Exp* 9: 133 (1934).

²⁶ Also the properties A and B in materials not of human origin are in general qualitatively different from the human A and B (cf. page 234).

²⁷ *Ztschr f indukt Abstamm u Vererbungs* 70: 463 (1936). However see Ottensooser and Tobler *Zeitschr f Immunstats* 90: 63 (1937). For a discussion of the question of independent inheritance of agglutinins cf. page 270.

The blood groups differ from many other characteristics in their independence of environmental influences. Once the blood groups are completely established (within the first two years of life) they remain essentially unaltered. This indeed is the basis of their medicolegal applications (cf. Chapters XXI and XXII).

Exceptionally an apparent change in blood group is seen which is produced by the transfusion of blood of another group. Thus in a case seen by the author an infant of group O was given blood of group AB through an error in typing (cf. page 4). No reaction resulted and the transfused blood could be demonstrated in the patient's circulation after the transfusion. Subsequent to the transfusion by the usual methods of blood grouping the child might have been considered to belong to group AB. This of course is not a true change in blood group and after the transfused blood disappears from the circulation the original group once more becomes apparent.²²

A number of authors have claimed to have observed changes in blood groups resulting from infectious diseases, X rays, galvanization, therapy with quinine, narcotics, etc. These results were certainly due to errors in technic; some of the investigators made use of a technic which favors errors, namely the mixture of whole blood or of corpuscle sediment with diluted serum (cf. page 20). In this connection it has been found that galvanization, X rays, and other agents influence the sedimentation time, so that some of the earlier observations were probably caused by rouleaux formation. Furthermore, in no case was the grouping confirmed by examination of the serum.

Similar investigations by reliable workers²³ on the other hand have failed to reveal any evidence for the belief that the blood groups can change.

Blood Groups in Twins. Two types of twins are distinguished: the uniovular type (identical twins) and the biovular type. Since uniovular twins arise by the division of a single ovum, their genotypes are identical, whereas biovular twins are no more closely related than ordinary brothers and sisters. It follows that the blood groups of uniovular twins must be identical, and such has been found to be the case. More recently, Schiff and Verschuer²⁴ have demonstrated that the genotypical identity of uniovular twins also holds for the agglutinogens M and N (cf. Chapter XIV) and the subgroups of group A and group AB (cf. Chapter XII). Therefore the agglutinogens of human blood can be used as an additional criterion for determining whether or not

²² Also see the interesting case reported by Lauer [*Ztschr. f. Immunitäts* 99: 433 (1941)].

²³ Schiff, *Technik der Blutgruppenuntersuchung*, p. 7, Berlin (1932); Landsteiner, Lattes, Hirsfeld, Snyder, personal observations, etc.

²⁴ *Klin. Woch.* 10: 723 (1931); *Ztschr. f. Morphol. u. Anthropol.* 32: 244 (1933).

twins are identical, alongside of sex, color of the eyes and hair, features, etc.⁴⁰ (cf. page 376).

Blood Groups and Sex Numerous studies have failed to show a significant difference in the distribution of the blood groups in the two sexes in the general population, or in the progeny of any of the ten possible matings. This indicates the absence of any relationship between blood groups and sex, and shows that the genes determining the blood groups are not located in the sex chromosomes (cf. page 166).

Blood Groups and Superfecundation Superfecundation, if it occurs at all, is admittedly a very rare phenomenon. Augsberger⁴¹ has suggested that this obscure question may be cleared up by means of blood grouping. Thus, according to Augsberger, if a group O mother gives rise to twins, one belonging to group A and the other to group B, if it is found that one father belongs to group A (or B), then there must be a second father belonging to group B (or A). A difficulty is the possibility that both twins could also be explained by adultery with a man of group AB. The same objection applies to the other combinations tabulated by Augsberger and Lattes,⁴² which, if they occur, are supposed to prove superfecundation. However, should a group O mother give rise to triplets belonging to groups O, A, and B, respectively, this anomaly could only be explained by assuming the existence of two fathers, so that superfecundation would be established. There are other similar cases which could prove superfecundation if tests are made for the subgroups of groups A and AB, e.g., group O mothers with triplets O, A₁ and A₂.

⁴⁰ Rife, *Jour Heredity* 24: 339 (1933), Wiener, *Human Biology* 7: 222 (1935)

⁴¹ *Klin Woch* 6: 1992 (1927)

⁴² *Individuality of the Blood*, p. 258

CHAPTER XII

THE SUBGROUPS OF GROUP A AND GROUP AB

BECAUSE the existence of subgroups in group A and group AB plays a rôle in the technic of blood grouping, we have found it necessary to refer to these subgroups a number of times in the preceding pages. In 1910, von Dungern and Hirsfeld¹ reported that when a group B serum was absorbed with certain group A bloods until it lost the power of agglutinating the absorbing blood, the serum still agglutinated most other bloods of group A and group AB. On the basis of these observations, they suggested a subdivision of group A and group AB. Similar observations were reported by Guthrie and Huck,² Coca and Klein,³ and Kline, Ecker and Young⁴ more than ten years later.

The existence of subgroups in group A and group AB depends on the fact that the agglutinin A is not a single substance, but includes two main sorts of properties, designated by Landsteiner and Levine⁵ as A_1 and A_2 . Thus there are two different sorts of group A blood, depending on whether the A agglutinin is A_1 or A_2 , and similarly there are two sorts of AB blood, A_1B and A_2B . As was pointed out in chapter II, the serums of group B and group O individuals contain agglutinins that react specifically with the agglutinin A. According to the older concept this isoagglutinin is a single entity designated as anti A or α . As a matter of fact, it has been found that the so-called α agglutinin is composed of qualitatively different fractions of which there are two main varieties: (1) anti A agglutinin proper, reacting with both agglutinogens, A_1 and A_2 , with approximately equal intensity, (2) agglutinin anti- A_1 , or α_1 , which reacts with agglutinin A_1 but practically not at all with agglutinin A_2 .

Different B and O sera contain these two agglutinin fractions in varying quantities and proportions. It is clear, therefore, that two different B sera which react with A_1 cells at approximately the same titer may have different titers when tested against A_2 cells. Thus, one serum, if it contains very much anti A_1 and very little of the common anti-A agglutinin, might have a titer of 40 for A_1 cells and only a titer of 5 for A_2 cells. On the other hand, another serum which contains a large proportion of common anti-A agglutinin might have a titer of 20 to 40 for A_2 cells even though the titer for A_1 cells is no greater than that of the first serum (40). In extreme in-

¹ *Ztschr f Immunitäts* 8: 526 (1911)

² *Bull Johns Hopkins Hosp* 34: 37, 80, 128 (1923)

³ *Jour Immunol* 8: 477 (1923)

⁴ *Jour Immunol* 9: 595 (1925)

⁵ *Jour Immunol* 18: 87 (1930)

stances B serum may react distinctly on A_1 and practically not at all on A_2 cells. This is the reason why every group B serum used for grouping purposes should be titrated against A_2 cells as well as A_1 cells (cf page 18). Since A_2 makes up approximately $\frac{1}{4}$ to $\frac{1}{3}$ of all group A individuals it is clear that with weak group B testing sera 20-25 per cent of group A individuals may be erroneously diagnosed as group O. In group AB errors in grouping are even more likely to occur. A_2B blood often being diagnosed as group B (cf page 35).

TECHNIC OF DIFFERENTIATING SUBGROUPS A_1 AND A_2

The simplest method of subgrouping group A and group AB blood is with the aid of so called "absorbed B serum."

Preparation of Absorbed B Serum This is a relatively simple procedure, being based on the fact that practically all group B sera contain two sorts of anti A agglutinins, namely, the common anti A agglutinin which acts on both A_1 and A_2 and anti A_1 which reacts only with A_1 . When the common anti A agglutinin is removed from such sera, only that isoagglutinin remains which reacts with A_1 cells so that in tests with such a reagent if agglutination occurs the blood belongs to subgroup A_1 (or A_1B), and if no clumping occurs the subgroup is A_2 (or A_2B). The reagent is prepared as follows. (If it is intended to preserve the reagent all the procedures are to be carried out under sterile precautions.) Blood is drawn from an individual of subgroup A_2 , washed twice with saline solution packed by centrifuging and the supernatant fluid is removed. Serum from a selected B individual (if fresh) is put in a water bath at 56°C for 10 minutes in order to inactivate it and then mixed with $\frac{1}{4}$ its volume of the packed washed A_2 cells. The mixture is allowed to stand for $\frac{1}{2}$ to one hour at room temperature and then centrifuged to separate the serum. As a rule, it will now be found that the serum no longer reacts with A_2 or A_2B bloods, but still agglutinates A_1 and A_1B bloods distinctly, otherwise the absorption should be repeated adding smaller quantities of A_2 cells. It is convenient to distribute the absorbed B serum in 1 c.c. ampoules, which are sealed and then placed in a water bath at 56°C for 20 minutes. These are stored in the refrigerator, and properly prepared reagents retain their potency under these conditions for at least a year.

If an absorbed B serum is not at hand individuals of subgroup A_1 can be selected by testing a series of group A bloods with a group B sera of different titers. Those group A bloods which give the weakest reactions, especially with sera of low titer, will usually prove to belong to subgroup A_2 . It should be pointed out also that not all group B sera, even those of high titer, yield satisfactory reagents, because the sera differ in their content of anti A_1 agglutinins. Usually those sera will be most suitable which on the direct tests intensely agglutinate A_1 blood but give distinctly weaker reactions with A_2 blood. If in doubt, one should first carry out a preliminary absorption experiment.

TABLE 28
FREQUENCY OF SERA CONTAINING THE IRREGULAR ISOAGGLUTININS α_1 AND α_2

Investigators	Number of Bloods Tested	Blood of Group	Number of Bloods in Group	Subgroup	Number of Bloods in Subgroup*	Number of Bloods Containing Irregular Isoagglutinins
Landsteiner and Witt		AB	30	$\left\{ \begin{matrix} A_1B \\ A_2B \end{matrix} \right.$	about 20 about 10	$A_1B\alpha_2$ 0 $A_2B\alpha_1$ 4
Matta	1154	$\left\{ \begin{matrix} A \\ AB \end{matrix} \right.$	413 100	$\left\{ \begin{matrix} A_1 \\ A_2 \end{matrix} \right.$ $\left\{ \begin{matrix} A_1B \\ A_2B \end{matrix} \right.$	about 330 about 83 about 67 about 33	$A_1\alpha_2\beta$ 2 $A\alpha_1\beta$ 7 $A_1B\alpha_2$ 2 $A_2B\alpha_1$ 7
Wiener	819	$\left\{ \begin{matrix} A \\ AB \end{matrix} \right.$	307 40	$\left\{ \begin{matrix} A_1 \\ A_2 \end{matrix} \right.$ $\left\{ \begin{matrix} A_1B \\ A_2B \end{matrix} \right.$	about 244 about 63 27 13	$A_1\alpha_2\beta$ 0 $A\alpha_1\beta$ 0 $A_1B\alpha_2$ 0 $A_2B\alpha_1$ 4

* The figures given here were mostly estimated from the known distribution of the subgroups (cf table 73) Also see Taylor, Race, Prior and Ikin *Jour Path and Bacteriol* 54 514 (1942)

Anti A_1 agglutinins can also be obtained by treating group O serum with A_2 cells, absorbed O serum, however, cannot be used for subgrouping group AB blood

The Tests These are carried out just like the ordinary grouping tests, either in tubes or on open slides, merely by mixing one drop each of blood suspension and of the absorbed B serum A convenient method is to perform the tests on a Boerner well slide as described for ordinary blood grouping (cf page 16) With good absorbed B sera, all A_1 and A_1B bloods give marked macroscopic clumping while A_2 and A_2B bloods show no trace of agglutination even upon microscopic examination It will be noticed, however, that while almost all A_1 bloods give complete or almost complete clumping with potent sera (with few or no free cells), A_1B bloods almost always are only partially clumped, about one tenth to one third of the cells remaining unagglutinated The significance of this observation still remains to be determined

A number of other methods of subgrouping group A and group AB bloods have been suggested The diagnosis can be made with the aid of the so called irregular isoagglutinins α_1 and α_2 which are not readily available however As has already been pointed out Landsteiner and Witt⁶ and Landsteiner and Levine⁷ showed that occasional sera from A_2 and A_2B bloods contain agglutinins which react specifically with bloods A_1 and A_1B while rare sera from A_1 and A_1B individuals contain agglutinins (α_2) reacting with A_2 blood Sera of the former type give identical reactions with the absorbed B sera and are therefore also designated as α_1 The so called α_2 agglutinins are not specific for A_2 since they also react with all group O bloods even more intensely than with A_2 cells and are therefore better known as anti O agglutinins The anti O (α_2) agglutinins moreover do not react regularly with A_2B bloods and also give weak reactions with certain B and A_1 bloods

One disadvantage of sera containing irregular isoagglutinins is that the reactions are usually not intense Moreover the irregular isoagglutinins as a rule are affected more by temperature changes than the absorbed B sera

Sera containing α_1 isoagglutinins are far more common than sera containing α_2 isoagglutinins, and irregular isoagglutinins are more frequent in group AB than in group A (cf table 28) As many as $\frac{1}{3}$ to $\frac{1}{5}$ of the bloods of subgroups A_2B have the constitution A_2Ba_1 while only about 1 out of 250 A_1 bloods has the irregular isoagglutinin α_2

Sera containing the agglutinin α_2 (or anti O) are more readily obtained from animals being most common among cattle and fowl Such sera cannot be used directly because they usually contain species agglutinins reacting with human blood of all groups However as Schiff⁸ showed bovine sera after absorption with blood cells A_1 or A_1B react specifically with bloods of group O and as was later pointed out also with bloods of subgroup A_2 The so called anti E agglutinin in eel serum is most likely related to the anti O agglutinin (cf page 264)

Even using all the reagents available the results are not always clean cut In an extensive study by Friedenreich and Zacho⁹ it was found that in more than

⁶ *Jour Immunol* 11 203 (1926)

⁷ *Jour Immunol* 12 441 (1926)

⁸ *Klin Woch* 6 303 (1927)

⁹ *Ztschr f Rassenphysiol* 4 164 (1930)

95 per cent of the bloods tested by the combined use of absorbed group B sera and sera containing the agglutinin α_2 the subgroup could readily be determined. About 5 per cent of the bloods failed to react with either serum or reacted with both sera. Bloods of this nature had been previously described by Landsteiner and Levine¹⁰ and were termed 'intermediates'. Subsequently Friedenrich and Zacho studied these bloods more thoroughly from the serological and genetic standpoint and found that bloods of group A reacted as follows:

95 per cent	$\begin{cases} +\alpha_1 \\ -\alpha_2 \end{cases}$	$\begin{cases} -\alpha_2 \\ +\alpha \end{cases}$	$\begin{matrix} A_1 \\ A_2 \end{matrix}$
5 per cent	$\begin{cases} +\alpha_1 \\ -\alpha_1 \end{cases}$	$\begin{cases} +\alpha_2 \\ -\alpha_2 \end{cases}$	$\begin{matrix} \text{most probably } A_1 \\ \text{most probably } A_2 \end{matrix}$

Since the α_2 agglutinin often does not react with A_2B bloods, only absorbed B sera can be used for subgrouping AB blood. In that case the diagnosis is made as indicated below:

$\begin{matrix} +\alpha_1 \\ -\alpha_1 \end{matrix}$	$\begin{matrix} A_1B \\ \text{most probably } A_2B \end{matrix}$
--	--

The agglutinin α (anti O) can also be present in immune sera. Thus as has been mentioned previously (cf page 31) Hooler and Anderson obtained one immune serum reacting apparently specifically with group O blood. One such serum was obtained by the present writer from a rabbit injected with human O blood. This serum when diluted 15 times with saline solution and absorbed with a half volume of A_1 cells yielded a fluid whose reactions corresponded with agglutinin α_2 . Several such sera have also been obtained by Matta¹¹ by immunizing rabbits and goats with group O blood. In a later chapter (cf page 338) anti-O sera will be discussed that were produced by immunizing goats with Shiga dysentery bacilli.

Differentiation of the Subgroups of Group A in the Newborn Inasmuch as the agglutinogens have not reached their full development at birth it is not an easy task to differentiate the subgroups of group A (and group AB) in the newborn. According to Worsaae the relative sensitivity of A_1 and A_2 in adults and newborn can be represented as follows:

$$\text{Adult } A_1 > \text{newborn } A_1 > \text{adult } A_2 > \text{newborn } A_2$$

The determination of the subgroups is not entirely reliable for infant's blood since for example the A_1 receptor may be so weak that it is mistaken for A_2 , the true subgroup not becoming evident until the child is older. If the blood cells react with B serum which has been absorbed with A_2 blood then it must belong to subgroup A_1 . With AB blood the determination of the subgroup in newborn is even less reliable.

C.

NATURE OF THE SUBGROUP DIFFERENCES

As has already been pointed out, it is generally accepted that the alpha agglutinins in group O and group B serum consist of two major qualitatively different agglutinins, α and α_1 . With respect to the nature of the difference between cells of subgroups A_1 and A_2 Landsteiner's¹²

¹⁰ *Jour Immunol* 18: 87 (1930)

Publication No. 11 Faculty of Medicine Egyptian University 1937

Landsteiner and Witt *Jour Immunol* 11: 203 (1926) Landsteiner and Levine

Jour Immunol 12: 441 (1926)

view is that this depends on the existence of two qualitatively different agglutinogens A_1 and A ¹³

However, some authors, particularly Lattes,¹⁴ consider the difference between the subgroups merely quantitative, that is, caused by different amounts of the same agglutinin in the erythrocytes. Lattes and Cavazutti¹⁵ pointed out that if group B sera containing both agglutinins α and α_1 are mixed with a large excess of A_2 cells, all the agglutinins present in the serum will be removed. And, as Friedenreich¹⁶ has demonstrated, the same effect is produced when the serum is absorbed with a very small volume of A_1 cells, as when it is absorbed with larger quantities of A_2 cells (cf. table 29)

TABLE 29
THE VON DUNGERN AND HIRSZFELD PHENOMENON
(After Friedenreich)

Group B Serum	Blood Cells	Dilution of Serum							
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
a) Before Absorption	A_1	+++	+++	++	++	++	+	+	0
	A_2	+++	++	++	+	+	0		
b) After Absorption with 1/8 vol A_2	A_1	++	+(+)	+	+	(+)	0		
	A	0	0						
c) After Absorption with 1/256 vol A_1	A_1	++	+(+)	+	+	Tr	0		
	A_2	0	0						

On the other hand, as evidence for the qualitative nature of the difference between the two subgroups Landsteiner and Witt cite the following experiment. By the method of splitting off agglutinins (cf. page 27), two fractions of agglutinins were separated from group B serum. One of the agglutinin solutions (split off from blood of subgroup A) agglutinated both A_1 and A blood to nearly the same titer, whereas the other (containing only the agglutinin α_1) did not act on A_2 blood although agglutinating A_1 blood not much less than the first agglutinin solution. This corroborates the existence of two qualita-

¹³ The other interpretation that A_2 blood has a single agglutinin A while A_1 blood has an additional agglutinin (composition AA_1) seems less likely though simpler for explaining the reactions with group B and absorbed B serum (cf. Landsteiner and Witt *loc cit*)

¹⁴ *Individuality of the Blood* p. 72 (1932)

¹⁵ *Jour Immunol* 9: 407 (1924) cf. Mino *Munch med Woch* 71: 1129 (1924)

¹⁶ *Ztschr f Immunitäts* 71: 1 (1931)

tively different agglutinins α and α_1 . Furthermore, this experiment may best be explained by assuming the existence of two qualitatively different agglutinogens, A_1 and A_2 , as Landsteiner does. According to this hypothesis, A_1 reacts more strongly with α than with α_1 , and A_2 reacts less intensely with α than A_1 , and only feebly with α . This can be represented as shown in figure 52.¹⁷

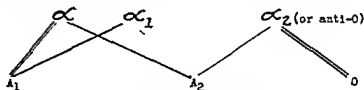


FIG. 52 (Modified after Friedenreich)

REACTIONS OF A_1 , A_2 AND O BLOOD WITH VARIOUS ALPHA AGGLUTININS

The theory does also satisfactorily explain why absorption with small quantities of A_1 blood produces the same result as absorption with larger quantities of A_2 blood.

As further evidence of the qualitative nature of the differences between A_1 and A_2 blood, Landsteiner and Levine¹⁸ point out that α_2 agglutinins react more strongly on A_2 than A_1 , in contrast to the reactions of α_1 agglutinins. That sera with α agglutinins reacted even more intensely with group O blood than with A blood (cf fig 52) was taken by these authors to indicate a relationship between the properties A_2 and O. According to Landsteiner's view, the composition of the subgroups would be represented as follows:

Group	Subgroups	Agglutinogens in Red Cells	Agglutinins Exceptionally Present in the Serum ¹⁹
A	$\{A_1$	A_1	(α)
	$\{A_2$	A_2	(α_1)
AB	$\{A_1B$	A_1 and B	(α)
	$\{A_2B$	A_2 and B	(α_1)

Thomsen,²⁰ however put forth the view that the so called agglutinin α_2 is really an agglutinin directed specifically to blood O and that it acts on A_2 cells only for the reason that they are mostly heterozygous (of genotype A_2O) and contain the factor O. This theory is supported by the fact that the α_2 sera agglutinate most intensely group O blood, and agglutinate A-B weakly, if at all. Furthermore, the failure

¹⁷ Friedenreich, *Ztschr f Immunstats* 71: 283 (1931).

¹⁸ *Jour Immunol* 17: 1 (1929).

¹⁹ Parentheses are used to emphasize that such (irregular) isoagglutinins are present only occasionally.

²⁰ Thomsen, *Acta Soc Medica Fenn A* 15: No 9 (1932).

of the serum to react with heterozygous A_1 bloods may be explained by assuming a dominance of A_1 over O . The occurrence of only a small percentage of weak reactions with group B blood can also be explained by assuming a partial dominance of B over O .

According to this explanation, moreover, none of the homozygous A bloods should react with the α agglutinin. It can be shown (cf. page 215) that the genotype A_2A_2 makes up $\frac{0.61}{10.05}$ or less than one six

teenth of all bloods of subgroup A and approximately 1.5 per cent of all group A bloods. By the same token a corresponding percentage of group A bloods should react with neither the absorbed B sera nor the sera containing the agglutinin α_2 and as has already been pointed out such bloods have actually been found. However, individuals of genotype A_1A_1 can be identified with certainty only if both parents are found to belong to subgroup A_2B . At any rate, Thomsen's explanation does not affect the arguments for a qualitative difference between the agglutinogens A_1 and A_2 , based upon the reactions with α and α_1 agglutinins.

The somewhat involved question of the reactions of anti O sera with homozygous and heterozygous bloods of groups A and B has recently been studied in detail by Dahr²¹ and by Hirsfeld²².

Further evidence that the difference between agglutinogens A_1 and A_2 is a qualitative one rather than quantitative one has been derived from direct titration experiments with human group B sera. As an example may be cited the recent study by Olbrich and Walther.²³ These investigators titrated 217 human B sera against A_1 , A_1B and A_2 and A_2B cells. The results with 5 selected sera are given below (the figures representing the titer for the respective test cells).

Test Cells	1	2	3	4	5
A_1	64	256	32	64	64
A_1B	32	64	—	—	—
A_2	8	16	16	32	8
A_2B	2	2	16	16	2

The fact that the ratios of the titers for A_1 and A_2 cells differ widely is proof of the qualitative nature of the difference between A_1 and A_2 . (The results also suggest the existence of some qualitative difference between the A agglutinogens in bloods A_2 and A_2B .)

Since A_1 and A_2 are qualitatively different it might be anticipated that group B (and group O) sera exist which contain anti A agglutinins reacting more intensely with A_2 than with A_1 cells. Such a serum has recently been found by the author (cf. table 30). The serum in question ($B \neq 4$) in contrast to other group B sera gave the stronger and quicker clumping with A_2 cells than with A_1 , though the end titers were about equal.

²¹ *Ztschr. f. Immunitäts* 92, 180 (1938).

²² *Schweiz. Ztschr. f. allgem. Path. u. Bakt.* 1, 23 (1938).

²³ *Ztschr. f. Immunitäts* 99, 194 (1941).

TABLE 30

COMPARISON OF REACTIONS OF DIFFERENT HUMAN GROUP B SERA WITH A₁ AND A₂ BLOODS

Serum No	Tested vs Cells	Dilution of Serum							
		Undil	1 2	1 4	1 8	1 16	1 32	1 64	1 128
B #1	O	-	-	-	-	-	-	-	-
	A ₁	+++	+++	+++	+++	+++±	++	+	-
	A ₂	+++	+++	+++	+++	+++±	++	+	-
	A ₂ B	+++	+++	+++	+++±	++	+	-	-
B #2	O	-	-	-	-	-	-	-	-
	A ₁	+++	+++	+++	+++±	++	++	+	-
	A ₂	+++±	+++±	++	+	-	-	-	-
	A ₂ B	++	+	-	-	-	-	-	-
B #3	O	-	-	-	-	-	-	-	-
	A ₁	+++	+++	+++	++	±	+	-	-
	A ₂	+++	++	+	-	-	-	-	-
	A ₂ B	-	-	-	-	-	-	-	-
B #4	O	-	-	-	-	-	-	-	-
	A ₁	+	+	+	+	±	-	-	-
	A ₂	±	±	±	+	±	-	-	-
	A ₂ B	++	++	++	±	+	-	-	-

It might also be expected that by immunizing rabbits with A₁ and A₂ bloods sera specific to each of the subgroups could be obtained. The few experiments made along this line have yielded inconclusive results however. In a recent study of an anti A₁ immune serum Wiener and Kosofsky²⁴ were able to demonstrate only a single variety of anti A agglutinin in the serum reacting more intensely with A₁ than with A₂ blood.

As has already been pointed out (cf page 31) Schiff and Adelsberger²⁵ found that by immunizing selected rabbits with human A blood sheep cell lysins are obtained due to the presence in human A blood and sheep cells of antigens related to the Forssman antigen designated F_A to indicate its presence in group A blood. Alune²⁶ and Klopstock²⁷ observed that A₂ cells have a weaker absorbing effect than A₁ cells on the sheep cell lysins in anti A₁ immune sera and concluded that the F_A component of the A antigen as well as the proper A component was less developed in A₂ blood than in A₁ blood (cf Thomsen²⁸). Moreover Elmenhoff Nielsen²⁹ observed that in the sera of patients of groups O and B with infectious mon-

²⁴ Jour Immunol 42 381 (1941)²⁵ Loc cit²⁶ Ztschr f Immunitats 73 75 (1931)²⁷ Ztschr f Immunitats 74 211 (1932)²⁸ Ztschr f Immunitats 87 335 (1936)²⁹ B. Elmenhoff Nielsen Bidrag til Belysning af Antigener i Menneskets Erythro-

onucleosis there develops a large difference in the titers for A_1 and A_2 cells and he even suggests that such sera are ideal for preparing α_1 agglutinins. After absorption with A_2 cells the sera still agglutinated A_1 cells strongly (titer 8-16) and the α_1 agglutinins in the sera were completely absorbed by treatment with sheep cells. An even more striking serum of similar type was encountered by Sachs³⁰ in a case of subacute bacterial endocarditis caused by a special strain of streptococcus viridans possessing antigens of the Forssman type. The reactions of this serum clearly demonstrate the qualitative difference between A_1 and A_2 cells.

Andersen³¹ immunized rabbits with A_1 and A_2 bloods and could demonstrate no difference in the behavior of the two sorts of immune sera in hemolysin tests with sheep blood.

HEREDITY OF THE SUBGROUPS

The first studies on the heredity of the subgroups were made by Landsteiner and Levine.³² In a later study,³³ these authors examined

TABLE 31
PHENOTYPES AND GENOTYPES OF THE SUBGROUPS

Phenotype	Genotype	
	Homozygous	Heterozygous
O	OO	
A_1	A_1A_1	A_1O and A_1A_2
A_2	A_2A_2	A_2O
B	BB	BO
A_1B		A_1B
A_2B		A_2B

69 families with 212 children and presented definite evidence of the hereditary nature of the subgroups. Thomsen, Friedenreich, and Worsaae³⁴ reported the results of a study of the subgroups in eleven families, and proposed a theory of heredity to explain their results. These authors postulate that instead of three allelic genes, A , B , and O (Bernstein's theory), there exist four allelic genes, A_1 , A_2 , B , and O , with genes A_1 , A_2 , and B dominant over gene O , and A_1 dominant over A_2 . The genotypes corresponding to the six possible phenotypes are given in table 31.

cyter af Type O A og AB mid speciel Undersøgelse af det ved Mononucleosis infectiosa (Monocytangina) forekommende heterogentiske Antistof (F Antistof). Kopenhagen 1936 (Danish).

³⁰ *Jour Path and Bact* 54 105 (1942)

³¹ *Ztschr f Rassenphysiol* 10 154 (1938). Cf Christensen *Acta Path et Microbiol Scand* 18 231 (1941)

³² *Proc Soc Exp Biol and Med* 24 941 (1927)

³³ Landsteiner and Levine *loc cit*

³⁴ *Acta Path et Microbiol Scand* 7 157 (1930), *Klin Woch* 9 67 (1930)

It is obvious that the theory of Thomsen, Friedenreich and Worsaae in no way contradicts and merely elaborates Bernstein's theory of heredity of the blood groups. According to their theory, the following additional rules of heredity should hold:

1 The agglutininogen A_1 cannot appear in the blood of a child unless present in the blood of one or both parents. This law depends upon the dominance of A_1 over A_2 . Thus in the matings, $A_2 \times A_2$, $A_2 \times O$, $A_1 \times B$, etc., all group A and AB children must belong to subgroups A_2 and A_2B , respectively. On the other hand, in the matings, $A_1 \times A_1$, $A_1 \times O$, etc., both A_1 and A_2 children may result, since the genotype of the A_1 parent could be A_1A_2 .

2 The combinations, A_1B parent with A_2 child, and vice versa, can not occur. For an A_1B individual can only produce germ cells bearing the gene A_1 , or bearing the gene B . The children must therefore possess at least one A_1 or one B gene and therefore cannot belong to subgroup A_2 (genotype A_2A_2 or A_2O). Similarly, A_2 cannot give rise to A_1B .

3 In the matings $A_1B \times B$ and $A_1B \times A_1B$, children of subgroup A_2B cannot result, because an A_1B individual cannot give rise to an A_2B child unless the other parent supplies the gene A_2 .

TABLE 32
HEREDITY OF THE SUBGROUPS

Parents	Children Possible	Children Excluded by Thomsen's Theory
1 $A_2 \times O$	O, A_2	A_1
2 $A_1 \times A_1$	O, A_1	A_2
3 $A_2 \times B$	O, A_2, B, A_2B	A_1, A_1B
4 $A_2B \times O$	A_2, B	A_1
5 $A_2B \times A_1$	A_2, B, A_2B	A_1, A_1B
6 $A_2B \times B$	A_2, B, A_2B	A_1, A_1B
7 $A_2B \times A_2B$	A_2, B, A_2B	A_1, A_1B
8 $A_1B \times O$	A_1, B	A_2
9 $A_1B \times A_1$	A_1, B, A_2B, A_1B	A_2
10 $A_1B \times A_2$	A_1, B, A_2B	A_1B, A_2
11 $A_1B \times A_2B$	A_1, B, A_2B, A_1B	A_2
12 $A_1B \times B$	A_1, B, A_2B	A_2, A_2B
13 $A_1B \times A_1B$	A_1, B, A_2B	A_2, A_2B
14 $A_1 \times O$	O, A_1, A_2	
15 $A_1 \times A_1$	O, A_1, A_2	
16 $A_1 \times A_2$	O, A_1, A_2	
17 $A_1 \times B$	$O, A_1, A_2, B, A_1B, A_2B$	
18 $A_1 \times A_2B$	A_1, A_2, B, A_1B, A_2B	

According to the theory of Thomsen, Friedenreich and Worsaae, therefore, the subgroups should be inherited as shown in table 32

4 Where several children are available in each mating, further special exclusions are possible in the following five crosses $A_1 \times O$, $A_1 \times A_1$, $A_1 \times B$, $A_1 \times A_1B$, and $A_1 \times A_2B$. In these matings, if there is one child belonging to either group O or group B, none of the remaining children can belong to subgroup A or subgroup A_2B , respectively, and conversely. For example, in the mating $A_1 \times O$, there are three possibilities, as follows

<i>Parents</i>	<i>Children</i>
$A_1A_1 \times OO$	A_1O
$A_1A_2 \times OO$	A_1O, A_2O
$A_1O \times OO$	A_1O, OO

It is clear, therefore, that if one child belongs to group O, the genotype of the A_1 parent must be A_1O , so that A_2 children cannot occur. Similarly, if there is one child belonging to subgroup A_2 , the genotype of the A_1 parent must be A_1A_2 , and group O children cannot occur.

The genotype of the A_1 parent can at times be defined if the groups of the grandparents are known. Thus, if one of the two grandparents belongs to subgroup A_2B , the A_1 parent must belong to genotype A_1A_2 . On the other hand, if one of the grandparents belongs to group O or group B, the genotype of the A_1 parent is A_1O . And if both grandparents belong to subgroup A_1B , the genotype of the A_1 parent is A_1A_1 . In all other cases, the genotype of the A_1 parent remains uncertain.²²

At the time when the former edition of this book was prepared not enough data had been accumulated to permit a definite conclusion as to the accuracy of the theory of Thomsen, Friedenreich and Worsaae. In recent years additional investigations on the heredity of the subgroups have been made, so that to date a total of 1068 families with 3134 children have been examined. The results obtained are summarized in table 33, and it will be seen that they strongly support the theory of 4 allelic genes. For example, in the families $A_1B \times O$, there were 55 children of subgroup A_1 and 53 of group B, corresponding almost exactly with predicted values, and none of subgroup A_2 , in conformity with the second rule of heredity, on the other hand, in the mating $A_2B \times O$, there were 37 A_2 and 27 B children, and none of subgroup A_1 , in agreement with the first rule. Moreover, not a single exception to the first rule was found among 155 additional children from matings $A \times B$, $A_2B \times A_2$ and $A_2B \times B$. Among the 331 children from the matings $A \times O$ and $A \times A$, however, there were 5 contradictions

²² Cf. Wolff and Jonsson, *Deutsch Zeitschr f d ges gerichtl Med* 22: 65 (1933).

TABLE 33

SUMMARY OF PUBLISHED STUDIES ON THE HEREDITY OF THE SUBGROUPS OF GROUP A AND GROUP AB

Parental Combination	Number of Families	Number of Children in Each Group						
		O	A ₁	A ₂	B	A ₁ B	A ₂ B	Totals
A ₂ × O	105	124	(4)	163	0	0	0	291
A ₂ × A ₂	13	13	(1)	26	0	0	0	40
A ₂ × B	43	21	0	34	29	0	32	116
A ₂ B × O	16	0	0	37	27	0	0	64
A ₂ B × A ₂	3	0	0	3	3	0	1	7
A ₂ B × B	10	(1)	0	10	15	0	6	32
A ₁ × O	387	404	695	69	0	(1)	0	1169
A ₁ × A ₁	168	79	394	16	0	0	(1)	490
A ₁ × A ₂	79	52	127	54	0	0	0	233
A ₁ × B	120	57	90	8	54	84	6	299
A ₁ × A ₂ B	16	0	14	9	10	8	2	43
A ₁ B × O	38	0	55	0	53	0	0	108
A ₁ B × A ₁	35	0	65	0	19	40	5	129
A ₁ B × A ₂	8	0	18	0	5	(1)	10	34
A ₁ B × B	25	0	15	0	32	16	0	63
A ₁ B × A ₁ B	2	0	6	0	2	8	0	16
Totals	1 068	751	1 484	429	249	158	63	3 134

Note The rare parental combinations, A₁B × A₂B and A₂B × A₂B have not been encountered in studies made to date

This table includes

- 103 families with 283 children by Friedreich and Zacho [*Ztschr f Rassenphysiol* 4 164 (1930)]
 32 families with 107 children by Schiff and Sasaki [*Ztschr f Immunitäts* 77 129 (1932)]
 89 families with 440 children by Wiener and Rothberg [*Human Biol* 5 577 (1933)]
 211 families with 309 children by Clausen [Undersøgelser over de Serologiske Blodtypegenskabe M og N, Copenhagen (1934)]
 71 families with 225 children by Maita [Publication No 11 of the Faculty of Medicine, Egyptian Univ (1937)]
 20 families with 46 children by Mustakallio [*Acta Soc Med Fenn 'Duodecim* 20 No 2 (1937)]
 30 families with 84 children by Moureau [*Ann de Méd Lég* 17 875 (1937)]
 106 families with 448 children by Dahr and Bussman [*Ztschr f Rassenphysiol* 10 49 (1938)]
 58 families with 138 children by Hirsfeld and Kostuch [*Schweiz Ztschr f allg Path u Bakteriol* 1 23 (1938)]
 73 families with 163 children by Taylor and Prior [*Ann Eng* 8 343 (1938)]
 38 families with 91 children by Worsaae [*Acta Path et Microbiol Scand Supp* 37 594 (1938)]

(Continued on opposite page)

to the heredity theory. Considering the large number of children examined, these are probably due to illegitimacy, and such is indeed the opinion of the investigators who reported these cases. In support of this conclusion one may also point to the occurrence of a group O child in the mating $A_2B \times B$ in contradiction to Bernstein's theory, and undoubtedly due to illegitimacy. Furthermore, two obviously illegitimate children occurred in matings $A_1 \times O$ and $A_1 \times A_1$.

Additional evidence in support of the theory of four genes is furnished by the families with one or both parents belonging to subgroup A_1B . In mating $A_1B \times A_1$, 65 of the 129 children belong to subgroup A_1 (number calculated from theory 64.5) and none to subgroup A_2 in conformity with the second rule of heredity. In mating $A_1B \times B$, there were 32 group B children (calculated number 31.5), 15 A_1 and 16 A_1B and none of subgroup A_2 or A_2B , in conformity with the second and third rules. Similarly, the distribution of the subgroups in the mating $A_1B \times A_1B$ conforms with the expectations. In the matings $A_1B \times A_2$, however, one child of subgroup A_1B was encountered, an exception to the second rule of heredity. This cannot be attributed to illegitimacy as it was the mother (not the father) who belonged to subgroup A_2 . Dahr and Bussman,⁷⁸ who found the case, ruled out technical errors by repeating the tests. The possibility that the child in question had been adopted could be considered. Another unexplained contradiction to the theory was reported by Thomsen, Friedenreich and Worsaae, namely, a family $A_2 \times O$ with two A_1 children. In this case, the authors suggested that the true phenotype of the parent might be A_1 , but that the sensitivity of the cells was weakened because of advanced age (80 years). Possibly this family actually contained individuals of the so called 'intermediate' subtype, the bloods being classified as A_1 for the children but as A_2 for the parents (cf. page 218).

As has just been pointed out, according to the 4 gene theory parents of subgroup A_1B cannot have A_2 children nor can A_2 parents have A_1B children. The theory can therefore be tested by examining a large series of mothers and children to de-

⁷⁸ *Ztschr f Rassenphysiol* 10: 49 (1938).

78 families with 301 children by Dahr [*Ztschr f Immunitäts* 97: 170 (1939)]

73 families with 238 children by Dahr, Offe and Weber [*Ztschr f Rassenphysiol* 11: 78 (1930)]

38 families with 153 children by Landsteiner and Wiener [*Jour Exp Med* 74: 309 (1941)]

To these totals are to be added the original study mentioned in the text of Landsteiner and Levine (69 families with 212 children) also a large pedigree consisting of 138 individuals and without a single contradiction to the theory has been reported by Thomsen [*Ztschr f Rassenphysiol* 5: 97 (1932)]

TABLE 34
SUBGROUPS OF GROUP A AND GROUP AB IN MOTHERS AND CHILDREN
(After Wolff and Jonsson)

Mothers	Children					
	O	A ₁	A ₂	B	A ₁ B	A ₂ B
182 O	111	35	17	19	—	—
190 A ₁	47	110	17	6	8	2
50 A ₂	16	10	22	0	—	2
58 B	15	3	4	32	2	2
15 A ₁ B	—	9	—	4	2	0
5 A-B	—	1	3	0	0	1
Totals	189	168	63	61	12	7

termine whether or not such combinations occur Wolff and Jonsson² did not find a single exception among the test cases contained in their series of 600 mother-child combinations (cf table 34) In the family data of table 33 as has just been mentioned one such case is included Further studies on mother-child combinations would accordingly be highly desirable

The 4 gene theory can be tested further by analyzing families in which one of the parents belongs to subgroup A₁ (cf rule 4) Inasmuch as individuals of this subgroup can belong to one of the three genotypes A₁A₁, A₁A₂, and A₁O, they can transmit gene A₁ alone or both A₁ and A₂ or both A₁ and O, but not all three genes, A₁, A₂, and O, to their children In families A₁ × O for example in some cases all the children will belong to subgroup A₁, in others half to subgroup A₁ and half to O, and in still others half to subgroup A₁ and half to subgroup A₂, families of the last type being the least common In table 35 we have compiled from the published investigations on the heredity of the subgroups all families A₁ × O in which there was at least one A₁ child Among the 31 families with 122 children listed, there were only 3 families containing any group O children and therefore in conflict with the theory The investigators who encountered these aberrant families believe that the legitimacy of the children in question may be doubted, and it is significant that one of the families also contained an A₁B child even though the blood of neither parent contained the agglutininogen B

In the A₁ × O families with A- children, it might be expected that half the children should be A₁ and half A₂ Actually, there is a slight deviation from the 1:1 ratio due to selection, inasmuch as families A₁A₂ × O in which by chance all the children belong to subgroup A₁ were excluded In order to determine how closely the observed distribution fits with the predictions, it is necessary to allow for the omitted

² *Deutsche Ztschr f d ges gerichtl Med* 22 65 (1933)

TABLE 35

LIST OF FAMILIES $A_1 \times O$ WITH ONE OR MORE CHILDREN OF SUBGROUP A-
(Compiled from the published studies on the heredity of the subgroups)

Investigators	Family Number	- Number of Children of group				
		O	A_1	A_2	Other	Totals
Schiff and Sasaki	22	0	4	1	0	5
	47	0	5	2	0	7
	50	0	2	3	0	5
Landsteiner and Wiener	11	0	2	3	0	5
	27	0	2	3	0	5
	54	0	3	3	0	6
Dahr and Bussman	6	1	3	3	1 A_1B	8
Dahr	9	0	2	4	0	6
Taylor and Prior	49	0	0	4	0	4
	50	0	4	3	0	7
Wiener and Rothberg	6	0	1	2	0	3
	109	2	4	1	0	7
	30	0	0	2	0	2
	33	0	1	2	0	3
Clausen	160	0	1	1	0	2
	196	0	1	1	0	2
	259	0	1	1	0	2
	272	0	1	1	0	2
Dahr, Offe and Weber	32	0	1	4	0	5
	43	0	2	2	0	4
	57	0	3	1	0	4
	64	2	1	2	0	5
Hirszfeld and Kostuch		0	1	1	0	2
Matta	26	0	1	2	0	3
	40	0	2	2	0	4
Mustakallio	13	0	0	2	0	2
Friedenreich and Zacho	8	0	2	1	0	3
	15	0	1	1	0	2
	26	0	1	1	0	2
	91	0	1	1	0	2
Wiener (unpublished)		0	1	2	0	3
Totals	31 families	5	54	62	1	122

TABLE 36
STATISTICAL ANALYSIS OF DATA IN TABLE 35

<i>s</i>	<i>n_s</i>	<i>sn_s</i>	$1-q^s$	$\frac{sn_s}{1-q^s}$	A ₁ expected $p \frac{sn_s}{1-q^s}$	A ₂ observed <i>r_s</i>
2	10	20	0.75	26.67	13.3	12
3	5	15	0.875	12.14	8.6	9
4	4	16	0.938	17.27	8.6	9
5	5	25	0.969	26.83	13.4	14
6	2	12	0.984	12.20	6.1	7
7	2	14	0.992	14.11	7.1	5
Totals	28	102		114.22	57.1	56

* Not including families containing contradictions to the theory

families, and this can be done, for example, with the aid of the formulae derived by Hogben.²² As is shown in table 36 the observed frequencies of the subgroups among the children closely agree with those calculated from the 4 gene theory. Statistical analysis of matings $A_1 \times A_1$, $A_1 \times B$, $A_1 \times A_1B$ and $A_1 \times A_2B$ yield equally satisfactory agreement between observation and expectation,²³ so that these findings strongly support the theory.

In view of all this evidence there can be no doubt regarding the accuracy of the theory of Thomsen, Friedenreich and Worsaae in principle, despite the existence of one or two conflicting cases (cf. also page 390). A complicating factor that has not been taken into account in the studies reported thus far is the existence of occasional bloods intermediate in their reactions between A_1 and A_2 , and possibly representing a special sort of A factor determined by a corresponding gene (cf. page 218). Probably some of the reported contradictions to the theory of Thomsen, Friedenreich and Worsaae were caused by the artificial classification as A_1 or A_2 of such bloods of intermediate type. In families where the presence of individuals of the rare intermediate type has been excluded, there appears to be no doubt that the subgroup differences are transmitted in conformity with the four gene theory.

Statistical Considerations. Let p_1 , p_2 , q , and r represent the frequencies of the genes A_1 , A_2 , B , and O respectively. If the frequencies of the six phenotypes in a homogenous population are known the frequencies of the genes can be derived as follows (cf. page 186)

²² *Jour. Genetics* 25: 97 (1931)

²³ The number of such families is small, however.

Phenotype	Genotype	Frequencies	
\overline{O}	OO	r^2	(1)
$\overline{A_1}$	$\begin{cases} A_1A_1 \\ A_1A_2 \\ A_1O \end{cases}$	$\begin{cases} p_1^2 \\ 2p_1p_2 \\ 2p_1r \end{cases} = p_1^2 + 2p_1p_2 + 2p_1r$	(2)
$\overline{A_2}$	$\begin{cases} A_1A_2 \\ A_2O \end{cases}$	$\begin{cases} p_2^2 \\ 2p_2r \end{cases} = p_2^2 + 2p_2r$	(3)
\overline{B}	$\begin{cases} BB \\ BO \end{cases}$	$\begin{cases} q^2 \\ 2qr \end{cases} = q^2 + 2qr$	(4)
$\overline{A_1B}$	A_1B	$2p_1q$	(5)
$\overline{A_2B}$	A_2B	$2p_2q$	(6)
and $p_1 + p_2 + q + r = 1$			7

It therefore follows that⁴⁰

$$p_1 = \sqrt{\overline{O} + \overline{A_1} + \overline{A_2}} - \sqrt{\overline{O} + \overline{A_2}} \quad (8)$$

$$p_2 = \sqrt{\overline{O} + \overline{A_2}} - \sqrt{\overline{O}} \quad (9)$$

$$q = \sqrt{\overline{O} + \overline{B}} - \sqrt{\overline{O}} \quad (10)$$

$$\text{and } r = \sqrt{\overline{O}} \quad (11)$$

For example, in Thomsen's data on the subgroups of 390 individuals (as calculated by Wellisch)

Group	O	A ₁	A ₂	B	A ₁ B	A ₂ B
Absolute	162	139	42	32	10	5
Per cent	41.54	35.64	10.77	8.21	2.56	1.28

$$\text{Therefore } p_1 = \sqrt{87.95} - \sqrt{52.31} = 21.46 \text{ per cent}$$

$$p_2 = \sqrt{52.31} - \sqrt{41.54} = 7.87 \text{ per cent}$$

$$q = \sqrt{49.75} - \sqrt{41.54} = 6.08 \text{ per cent}$$

$$r = \sqrt{41.54} = 6.45 \text{ per cent}$$

$$\text{and } p_1 + p_2 + q + r = 100.07$$

which corresponds satisfactorily with the expectations

The values obtained above for p_1 , p_2 , q and r with the aid of equations 8 through 11 when substituted in relations 1 through 4 in order to recalculate the frequencies of O , A_1 , A_2 and B will naturally give an exact fit since these four equations were used to derive formulae 8 through 11. However, by substituting the calculated values of p_1 , p_2 and q in equations 5 and 6 one can determine how closely the actual frequencies of subgroups A_1B and A_2B conform with those expected under the heredity theory. This has been done below and it will be seen that despite the small number of group AB individuals tested (15) the fit is a good one

$$\overline{A_1B} = A_1B = 2p_1q = 2.62$$

$$\overline{A_2B} = A_2B = 2p_2q = 0.95$$

Stevens⁴¹ has applied Fisher's method of maximum likelihood for the estimation of the values p_1 , p_2 , q and r , because, as he points out, the values given by relations

⁴⁰ See Wellisch and Thomsen, *Hereditas* 14: 50 (1930)

⁴¹ *Ann. Eug.* 8: 362 (1938)

(8) to (11) are inefficient. Applying his method to the data of Taylor and Prior⁴² then recalculating the frequencies of the groups and subgroups from the frequencies of the genes and applying the χ^2 test Stevens obtained an excellent confirmation of the four gene theory ($\chi^2=2.18$ for two degrees of freedom so that $P=35\%$ approximately). For the method of making these complicated calculations the reader is referred to the original paper by Stevens.

Wolff and Jonsson⁴³ have tested Thomsen's theory statistically with the aid of the relation $p_1/p_2 = A_1B/A_2B = A_1 \text{ children}/A_2 \text{ children}$ (from mothers of group O). This test has also been used by Mustakallio⁴⁴ (cf. table "3").

AGGLUTINOGEN A_3

Fischer and Hahn⁴⁵ and Friedenreich⁴⁶ independently reported the occurrence of individuals of group A whose blood gave only feeble reactions even with the most potent anti A sera. Friedenreich attributes the weak activity of such bloods to a third sort of A agglutino-gen, designated as A_3 . The existence of agglutino-gen A_3 gives rise to two additional subgroups in group A and group AB namely subgroups A_3 and A_3B , respectively.

The properties of blood of subgroups A_3 and A_3B have been studied in detail by Friedenreich. These subgroups can be recognized without using any special method by their weak reactions with anti A sera. Like A blood A_3 blood is agglutinated by sera containing α_2 (anti O) agglutinins. The ability of A_3 blood to absorb anti A agglutinins is intermediate between that of A_1 and A_2B blood. A_3 sera contain β isoagglutinins just like ordinary group A sera but (as in A_2 sera) α_1 cold agglutinins may be present in these and in A_3B sera.

Bloods of subgroups A_3 and A_3B are rare but by testing a series of 4000 to 5000 individuals Friedenreich found 6 such cases. 260 members of the families of these 6 persons were tested and an additional 46 A_3 and A_3B individuals were found. It is evident therefore that the occurrence of the property A_3 is determined by heredity. The pertinent matings in Friedenreich's study are summarized as follows:

Matings	Total Number of Children	Number of Children of Group		
		O	A_1	A_3
$A_3 \times O$	30	15	0	15
$A_3 \times A_1$	22	4	12	6

As Friedenreich believes his findings indicate the existence of a fifth allele A_3 in addition to the genes O, A_1 , A_2 and B already postulated. According to Friedenreich the gene i is recessive to genes A_1 and A_2 but dominant over gene O.

Three blood samples were recently submitted to the author for examination on account of their weak reactions in anti A sera and these proved to correspond in their behavior with Friedenreich's subgroup A_3 . The reactions of one of these bloods in anti A sera are shown in figure 33. The characteristic feature of the agglu-

⁴² *Ann. Eng.* 8: 344 (1938).

⁴³ *Loc. cit.*

⁴⁴ *Acta Soc. Med. Fenn. "Duodecim"* 20: No. 2 (1937).

⁴⁵ *Ztschr. f. Immunitäts* 84: 177 (1935).

⁴⁶ *Klin. Woch.* 15: 310 (1937); *Ztschr. f. Immunitäts* 89: 409 (1936).

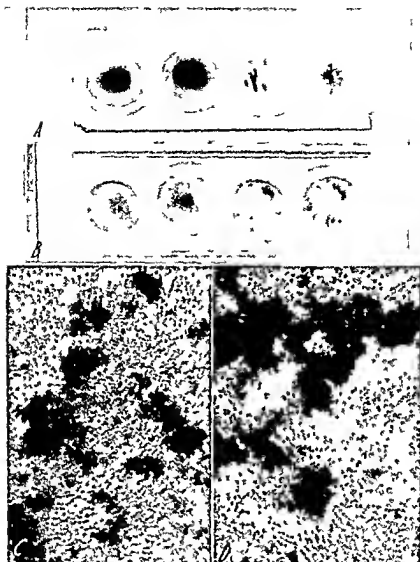


FIG 53 AGGLUTINATION REACTIONS OF AN A_2 BLOOD WITH TWO POTENT ANTI A SERA

A and B Actual size The first and second well of each slide show the reactions of the A_2 blood the third the reactions of an A_1 blood and the fourth the reactions of an A_2 blood

C and D Microscopic appearance Note the large number of unagglutinated cells

[Reproduced from the *Amer Jour of Clin Path* 11 45 (1941)]

tionation reactions is the weak and only partial clumping of the erythrocytes so that many cells remain unagglutinated even in high titered anti A sera that cause complete agglutination of A_2 blood The microscopic picture of the tests resembles that of patients of group A who have received transfusions of O blood. In one

of the author's cases⁴⁷ the serum of a woman contained the irregular isogglutinin α_1 and the blood of her only child one year of age behaved exactly like the patient's also possessing the constitution $A_2\alpha_1$.⁴⁸ In a second case⁴⁹ the bloods of a brother and sister both had the constitution $A_2\alpha_2$.

At the present time the main practical significance of the existence of the A_2 receptor is that its feeble agglutination reactions may lead to errors in diagnosis. With regard to the possibility of the existence of further variants of the A agglutinin, A_1 , A_2 , etc., cf. Friedenreich,⁵⁰ Hirsfeld and Amzel⁵¹ and Gammelgaard and Marcussen.⁵²

As has already been mentioned Landsteiner and Levine encountered occasional bloods which gave reactions intermediate between A_1 and A_2 . Such bloods have also been encountered by other investigators (Friedenreich and Zacho, Hirsfeld and Kostuch) including the present author. For the sake of simplicity these few exceptional bloods were not included in the general heredity table (cf. table 33). As to the nature of these intermediates it is significant that such bloods are usually familial indicating that they represent a special variety of A agglutinin determined by a corresponding special gene.⁵³ Further investigation of this problem would be desirable.

The practical importance of the so-called intermediates is that with weak α_1 sera they would be diagnosed as A_2 while with stronger sera they might be classified as A_1 . In this way seeming contradictions to the theory of heredity proposed by Thomson, Friedenreich and Worsaae could arise.

SUBGROUPS OF GROUP B

Numerous unsuccessful attempts have been made to demonstrate the existence of subgroups in group B, analogous to those in group A, by absorbing group A sera with various group B bloods and testing the absorbed sera against other group B bloods. As an example the experiments of Worsaae⁵⁴ may be cited. However, from a number of recent reports the existence of differences in agglutinin B would not seem improbable.⁵⁵

The investigations on differences in agglutinogens B, and their supposed hereditary nature, and irregular isogglutinins analogous to α_1 and α_2 , have not yet reached the stage where practical application is possible. For further information reference may be made to the study by Matta.⁵⁶

⁴⁷ Wiener and Silverman *Amer Jour Clin Path* 22: 45 (1931).

⁴⁸ Wiener unpublished observations.

⁴⁹ *Klin Woch* 16: 753 (1937).

⁵⁰ *Rev d Immunol* 6: 31 (1940).

⁵¹ *Ztschr f Immunstats* 98: 411 (1940).

⁵² Wiener unpublished observations. Landsteiner personal communication.

⁵³ *Ztschr f Rassenphysiol* 7: 17 (1935).

⁵⁴ Masaki, Kuwashima, Iseki and Fukao *Hanraigaku Zasshi* (Japanese) 9: 467 (1935), Iseki *Hanraigaku Zasshi* (Japanese) 10: 373 (1936).

⁵⁵ Matta, Publication No. 11, Faculty of Medicine of the Egyptian University (1937).

CHAPTER XIII

THE AGGLUTINOGENS M AND N OF LANDSTEINER AND LEVINE*

BY MEANS of the four blood groups including two subgroups in group A and group AB, all human beings can be divided into six classes. Later investigations have revealed the presence in human red blood cells of agglutinable factors unrelated to A and B, so that a further subdivision of human bloods is possible. In this chapter, two agglutinogens which have been thoroughly studied will be discussed, namely, the agglutinogens M and N.

In 1927, Landsteiner and Levine¹ observed that when certain immune sera from rabbits which had been injected with human blood were exhausted with certain samples of human blood, they still contained agglutinins acting on the majority of bloods of all four groups, while other bloods were not agglutinated. Two of the factors demonstrable by these sera were designated M and N.

These authors found that according to their content of agglutinogens M and N, three distinct types of human blood could be distinguished, type M (blood possessing agglutininogen M but lacking agglutininogen N), type N (blood possessing agglutininogen N but lacking agglutininogen M) and type MN (blood possessing both agglutinogens, M and N). Not a single blood lacking both agglutinogens was found. The distribution of the three types, M, N and MN is the same in each of the four blood groups so that the agglutinogens M and N are unrelated to the agglutinogens A and B.

TECHNIC

Preparation of Immune Sera. Among hundreds of thousands tested only seven human sera have thus far been encountered which contained anti M agglutinins,² none have been found with anti N agglutinins.³

* The author's studies on this subject were aided by grants from the Committee on Scientific Research of the American Medical Association.

¹ *Proc Soc Exp Biol and Med* 24: 600-941 (1927). *Jour Exp Med* 47: 757 (1928).

² In four of the seven cases the anti M isoagglutinins were presumably natural [Wolff and Jonsson *Deutsch Ztschr f d ges gerichtl Med* 22: 84 (1933); Friedenreich *Ztschr f Immunitats* 91: 485 (1937); Moureau and Lambert *Ann de Med Leg* 20: 163 (1940); Davidsohn and Schurmer *Proc Chicago Path Soc*, (unpublished) Oct. 13, 1941] in two cases they resulted from isommunization by blood transfusion [Wiener and Forer *Proc Soc Exp Biol and Med* 47: 215

The testing sera are usually obtained by immunizing rabbits. To produce anti M serum, blood of type M (group O), and to produce anti N serum, blood of type N (group O), should be used, so that no agglutinins are formed against A and B.

The technic used by Landsteiner and Levine consisted in weekly injections of fresh blood drawn into citrate and washed with saline before the injection. The first injection of 3 c.c. was given intravenously; the following injections of 4 c.c. each intraperitoneally. The immune rabbit sera were tested 6 days after the third and each subsequent injection. The animals were bled (mostly after four or five injections) the day following the tests when the sera had a sufficient content of the desired antibodies, i.e. when they gave powerful specific reactions after absorption.

The technic found most effective by the author⁴ is to alternate courses of daily intravenous injections of washed blood cells with rest periods. If twelve rabbits are to be immunized, 25 c.c. of blood will suffice for one course for all the rabbits. This blood may be stored in the ice box in the Rous mixture (cf. page 12) and is washed with normal saline before injection. All the injections of the first course are given intravenously. To avoid anaphylaxis the first injection of each of the subsequent courses may be given intraperitoneally or subcutaneously; the other injections are given intravenously. The rest periods between courses may be 7 days. The sera are tested one week after the last injection of a course. After three or four courses of injections, about half of the rabbits produce good anti M and anti N sera.

The sera are obtained under sterile precautions and stored preferably in small portions without the addition of any preservative. Some workers advise that the sera be passed through Berkefeld or Seitz filters to insure sterility. When some of the writer's anti M and anti N immune sera were examined after storage in the ice box for more than five years, no significant diminution in titer could be detected. Other sera, however, may lose their specific agglutinating power after a shorter time.

It may prove advantageous not to bleed the rabbit to death but after a rest period of a few months' duration to give an additional course of injections in order to stimulate the reappearance of antibodies. Moreover, by this method good sera can often be obtained in cases where the rabbit initially fails to form satisfactory antibodies.⁵

The writer has been able to obtain anti M and anti N sera by immunizing cats, but these sera were too weak to be of practical value. Landsteiner⁶ has obtained satisfactory anti M sera by immunizing goats, and Olbrich⁷ was able to induce the formation of N but not M antibodies in rats.

Wheeler, Sawin and Stuart⁸ have summarized the published results of immunizations of rabbits against human M and N erythrocytes.

(1941) Wiener *Amer Jour Clin Path* 12: 302 (1942).] Also see Paterson, Rice and Taylor *Brit Med Jour* 2: 37 (1942).

⁵Iseki and Fukao [*Han argaku Zasshi* (Japanese) 10: 657 (1936)] have described a normal rabbit serum containing anti N agglutinins. This is the only instance thus far reported of agglutinins for M or N in normal animal sera.

⁶*Amer Jour Med Sci* 186: 257 (1933).

⁷Wiener, Zinsser and Selkowitz *Jour Immunol* 27: 431 (1934). Cf. *Deutsch Ztschr f d ges gerichtl Med* 20: 316 (1933).

⁸Personal communication.

⁹*Ztschr f Immunitat* 91: 242 (1937).

¹⁰*Jour Immunol* 37: 159 (1939).

While the results differed somewhat depending on the rabbits and method of immunization used, in general, rabbits respond more readily to injections of type N blood than to type M blood. In fact, most workers, including the present author, have found that all or almost all rabbits are capable of producing some N antibodies, although only about two thirds of the sera yield reagents of sufficient potency for use. On the other hand, although Wheeler, Sawin and Stuart observed that almost three fourths of a series of 116 rabbits produced sera with

TABLE 37

SPECIFIC M AGGLUTININS IN 116 IMMUNE RABBIT SERA AFTER ABSORPTION WITH POOLED HUMAN ON AN AND BN CELLS
[Wheeler, Sawin and Stuart, *Jour Immunol* 37 159 (1939)]

Titer for Type M Cells of		Number of Rabbits	Per cent of Total
Stock Serum	Testing Fluid		
Less than 40	0	32	27.6
80	2	2	1.7
160	4	6	5.2
320	8	11	9.5
640	16	20	17.2
1 280	32	14	12.1
2 560	64	13	11.2
5 120	128	15	12.9
10 240	256	3	2.6

some M agglutinins only about two thirds of these sera were satisfactory for use (cf table 37). An important difference between the M and N antisera (cf page 222) is that while the former not infrequently yield testing fluids with titers as high as 128 or higher, the testing fluids from N antisera rarely have titers beyond the minimum acceptable one of 16.

Wheeler, Sawin and Stuart have attempted to explain the inability of some rabbits to produce M antibodies on a constitutional basis and from breeding experiments concluded that this is determined by a pair of allelic genes, *M* and *m*, the resistant rabbits possessing two recessive genes, *mm*. However, inasmuch as the mating *mm* × *mm* was not included in the study, this theory has not yet been subjected to a critical test. Attempts to breed a pure strain of rabbits capable of producing high titered M and N antisera have apparently not yet yielded decisive results.*

*With regard to qualitative differences in M and N sera which, however, are of no consequence for practical application cf page 343.

Preparation of Testing Fluids The sera that are obtained from the rabbits cannot be used directly since they contain, in addition to the specific agglutinin, species agglutinins acting on all human bloods. The species agglutinins must be removed by absorbing the immune serum with human red blood cells not containing the specific agglutino-gen in question.

The prime requirement for preparing strong and specific testing fluids is to start with immune sera of sufficient potency. If the original immune sera are weak, no artifice in the technic of absorption can completely compensate for the defect.

Unless the absorption is carried out properly, the testing fluids will be found unsuitable for use, even if one starts with a good immune serum. It is more difficult to prepare satisfactory N testing fluids than M fluids, the reason being as Landsteiner and Levine¹⁰ have pointed out, that when anti N sera are treated repeatedly with M blood the specific anti N agglutinins are gradually removed. On the other hand practically all anti M sera resist non specific absorption.

If the immune serum is fresh, it must first be inactivated¹¹. Then the serum is diluted 15 to 25 times (depending upon its strength) with normal saline solution and mixed with a half volume of packed washed cells of the proper type. After 30 minutes, the mixture is centrifuged and the supernatant fluid is pipetted off. With some immune sera a single absorption may suffice to remove the species agglutinins; most sera must be absorbed twice or more.

When preparing anti M fluids, the absorptions are best carried out at room temperature. Since there is little or no danger of non specific absorption, no damage is done if an excess of type N blood is added to the diluted immune serum in the process of preparing the testing fluids (cf. table 38). In any event, sufficient absorbing blood must be used to insure the complete removal of the species antibodies. When the absorptions are carried out at lower temperatures smaller amounts of blood are required, as a rule.

In the preparation of N fluids an excess of absorbing blood must be avoided lest the specific agglutinins be weakened. Hence the preparation of N fluids is best carried out slowly, in a number of steps, so that the point of complete absorption can be determined accurately. In order to obtain good results, moreover, the optimum dilution of the serum should be ascertained by preliminary tests. (In the author's experience, most potent N, as well as M, sera yield good testing fluids at a dilution

¹⁰ *Jour. Exp. Med.* 47: 769 (1928) *ibid.* 48: 734 (1928)

¹¹ With stored sera this is unnecessary since complement is destroyed after serum has remained in the ice-box for some time.

TABLE 38

PREPARATION OF SPECIFIC ANTI M TESTING FLUID

One drop of progressively doubled dilutions of testing fluid, one drop of cell suspension and one drop of normal saline solution were mixed in a series of tubes. After the mixtures had stood for 2 hours at room temperature the rack holding the tubes was shaken and the reactions read. Absorptions and titrations were done at room temperature.

Immune Serum Diluted 20×	Tested Against Cells	Dilution of Testing Fluid*									
		1 1	1 2	1 4	1 8	1 16	1 32	1 64	1 128	1 256	
Before absorption	M	+++	+++	+++	+++	+++	++	+	+	-	
	N	+++	+++	+++	+++	+++	+++	++	+	-	
	MN	+++	+++	+++	+++	+++	+++	++	+	-	
After first absorption with half volume of pooled N blood	M	+++	+++	+++	+++	++	++	+	±	-	
	N	++	±	-	-	-	-	-	-	-	
	MN	+++	+++	+++	+++	++	+	-	-	-	
After second absorption with half volume of type N blood	M	+++	+++	+++	+++	++	+	-	-	-	
	N	-	-	-	-	-	-	-	-	-	
	MN	+++	+++	+++	+++	±	-	-	-	-	
After third absorption with half volume of type N blood	M	+++	+++	+++	+++	±	±	-	-	-	
	N	-	-	-	-	-	-	-	-	-	
	MN	+++	+++	+++	+++	+	-	-	-	-	

* To obtain serum dilution, multiply dilution of testing fluid by 20

of about 1:20.) The absorptions can be made at room temperature, or according to some authors,¹² preferably at 37°C.

To illustrate the method of preparation of anti N testing fluids the following experiment with an N immune serum is cited. The serum was diluted 20 times with normal saline and then absorbed two times with half a volume of packed washed cells of type M at 37°C. The diluted serum was titrated before and after each absorption. It may be seen that after the first absorption most of the species agglutinin was exhausted (cf. table 39). After the second absorption the remainder of the species agglutinin but also a considerable portion of the specific anti N agglutinin was removed. After a third absorption the fluid completely lost its activity. It is evident that a more potent and specific N fluid can be obtained from this particular N serum if after the first absorption the procedure is continued.

¹² Landsteiner and Levine *loc. cit.*

more cautiously by using smaller amounts of M blood for each succeeding absorption, for example, $\frac{1}{2}$ or $\frac{1}{4}$ volume, instead of $\frac{1}{2}$ volume. Facility with the technic of preparing potent specific testing fluids especially for agglutinin N can only be acquired with experience.

In this connection the merit of the method suggested by Pietrusky¹³ for preparing testing fluids potent enough to detect the weak agglutinin N₂ (cf page 227) has not yet been established.¹⁴ Nor is there any convincing evidence that testing fluids prepared as suggested by Lattes by absorbing the stock immune sera with boiled erythrocytes are superior to fluids prepared in the usual manner. In

TABLE 39
PREPARATION OF ANTI N TESTING FLUID
(Absorptions at 37°C. Titrations at room temperature)

Immune Serum Diluted 20×	Tested Against Cells	Dilution of Testing Fluid						
		1:1	1:2	1:4	1:8	1:16	1:32	1:64
Before absorption	M	+++	+++	+++±	++	++	±	+
	N	+++	+++	+++±	+++	++	++	+
	MN	+++	+++	+++	+++	++	+	+
After first absorption with half volume of pooled M blood	M	+++±	±	-	-	-	-	-
	N	+++	+++	+++	++	±	tr	-
	MN	+++	+++	+++	++	++	-	-
After second absorp- tion with half vol- ume of type M blood	M	-	-	-	-	-	-	-
	N	+++	+++±	tr	-	-	-	-
	MN	++	±	-	-	-	-	-

fact since the capacity of boiled erythrocytes to absorb species agglutinins is considerably less than that of fresh red cells much more blood is required and that fluids prepared with boiled erythrocytes are colorless instead of pale pink is only a minor advantage.¹⁵

As a preservative the author employs merthiolate in the proportion of 1 part of a 1 per cent solution for every 50 parts of testing fluid. Stored in this manner in the refrigerator the anti M and anti N fluids are usually suitable for use for at least a year. The stability of these reagents seems to be a property of the particular immune serum from which they are prepared rather than to depend on the preservative added, those of high initial titer being most stable.¹⁶ Thus the author has some anti M fluids which react satisfactorily after 4 years of storage while good anti N fluids were usable for somewhat shorter periods. The use of toluol as a preservative in accordance with Levine's suggestion¹⁷ has the disadvantage that it causes the formation of a precipitate and the toluol should be removed before

¹³ *Deutsche Ztschr f d ges gerichtl Med* 28: 468 (1937)

¹⁴ *Cf* Clauberg *Klin Woch* 16: 1749 (1937)

¹⁵ *Elbel Ztschr f d ges gerichtl Med* 24: 242 (1935)

¹⁶ *Cf* Olbrich *Ztschr f Immunitat* 90: 271 (1937)

¹⁷ *Jour Lab and Clin Med* 26: 866 (1941)

the reagent is used (cf Boyd¹⁸). Boyd has used dyes such as acriflavine and brilliant green to preserve M and N fluids, and has also pointed out that if desired each fluid could be colored differently, as is often done with A and B grouping sera (cf page 11) for purposes of identification. While, as Boyd has shown, M and N testing fluids can be kept in a dry form after lyophilization in the Flosdorf-Mudd apparatus,¹⁹ it is doubtful whether this offers any advantage, considering the stability of the reagents in the more convenient liquid form.²⁰

The Tests. The actual tests are simple to perform, and if the testing fluids have been properly prepared, very easy to read. Any technic used for ordinary blood grouping is suitable for M-N tests. Probably the most convenient method is to set up the tests in small tubes (in-

TABLE 40
TESTS FOR M AND N BY CENTRIFUGE METHOD
(Family No. 92 of study by Wiener and Vaisberg*)

Blood of	Reaction for M	Reaction for N
Father	+++	+++
Mother	-	+++
First child	-	+++
Second child	-	+++
Third child	+++	++±
Controls Type M	+++	-
Type MN	+++	+++
Type N	-	++±

* *Jour Immunol* 20, 371 (1931)

side diameter 7 mm) by mixing one drop each of unknown cell suspension and testing fluid. Then after the mixture has stood for 1 to 2 hours at room temperature, it is examined both macro- and microscopically for agglutination.

An alternative technic is the centrifuge method. It has the advantage that the final reactions can be read within a few minutes. The tubes are centrifuged for a short time and then shaken until the negative control breaks up into an even suspension. If the unknown blood does not contain the agglutinin in question, the centrifuged cells at the bottom of the tube will disperse into a microscopically homogeneous suspension. If the unknown blood contains the agglutinin in question, the mass of centrifuged cells will remain as a single large clump, or break up into macroscopic clumps of agglutinated cells. At least one, preferably more, control blood cell suspension of each of the three types, M, N,

¹⁸ *Jour Immunol* 37, 65 (1939)

¹⁹ Cf. Jonsson *Acta Path et Microbiol Scand* 10, 438 (1933)

²⁰ Wiener, unpublished observations

and MN, should be included in every experiment. A sample protocol is given in table 40

By this technic the distinction between M+ and M— blood is always sharp. When testing for N, however, difficulty may occasionally be encountered. A diagnosis of N+ or N— can always be made in doubtful cases, however, if the tests are repeated a sufficient number of times, with several different sera.

The tests may also be set up by mixing cell suspensions and testing fluids on a glass slide, but this is not the method of choice. One drop of M fluid is placed on one side of the slide and one drop of N fluid is placed on the other. Then one drop of unknown cell suspension is mixed with each of the reagents. The slide is tilted back and forth for 5 to 10 minutes, after which time the drops may be covered with a cover slip to prevent drying. When many specimens are to be examined, well slides (cf. page 14) can be used.

Schiff²¹ recommends that in all cases where the reactions are not absolutely clean-cut, the results be controlled by testing the ability of the unknown blood to absorb the specific agglutinins. This additional control is certainly never necessary when testing for M with suitable sera. In the author's opinion, moreover, absorption tests could hardly be of much help where the reactions for N are not decisive, since as has been pointed out, blood not containing N, if used in excess, can absorb N agglutinins. The best control is the repetition of the tests with fresh blood and with several different anti-M and anti-N fluids, including adequate controls.

The Boerner well slides, which have 12 wells distributed in 3 rows of 4 wells each (cf. page 16) are convenient when it is desired to test bloods for the A B groups and M N types simultaneously. Three bloods can be tested on each slide. First one drop each of group A, group B, anti M and anti N sera are added to the 1st, 2nd, 3rd and 4th columns of wells, respectively. To each of the 4 wells in the first row is added one drop of the first blood suspension, to the second row of wells the second blood suspension is added, etc., and finally one drop of saline is added to every well. The slides are then shaken by hand or on the shaking machine for 10 minutes, and the reactions read.

FACTORS AFFECTING THE INTENSITY OF THE REACTION

Concentration of the Cell Suspension. As is the case when testing for the factors A and B, the concentration of the cell suspension used is of considerable importance. Highly concentrated suspensions require more antibody for agglutination than dilute ones, and may fail to react even with sera of medium potency. Consequently, the concentrations of the blood cell suspensions should be made uniform. The best results are obtained with 1 to 3 per cent suspensions.

Temperature. The avidity with which the immune species specific agglutinins combine with red cells is influenced by temperature, similar to the behavior of the isoagglutinins (cf. page 18). Thus, if anti-N immune sera are absorbed with type M blood until they no longer react with bloods of that type at a given temperature (e.g., room tempera-

²¹ *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 18: 57 (1931)

ture), it will often be found that such testing fluids still react with bloods not containing N, when the tests are performed at lower temperatures (e g, ice box temperature) Also the specific reactions, especially for N, are weakened by an increase in temperature These effects of temperature on the tests should be taken into account to avoid mistakes

Age of the Blood Cell Suspension When blood cells are kept in saline, there is a certain loss of sensitivity with time, which is least noticeable with blood stored at low temperatures Old blood cell suspensions should also be avoided because of an occasional tendency to spontaneous agglutination

The present author has found it possible to type blood which had been shipped long distances by mail, provided that the blood was taken under sterile precautions and properly put up When it is desired to store blood or ship it long distances by mail, the following procedure should be followed At least 1 to 2 cc of sterile venous blood are required Half of the blood is placed in one vial without the addition of any preservative, and the other half is mixed with Rous and Turner's solution as described on page 12

Sensitivity of Agglutinogens Landsteiner and Levine²² found that bloods containing factor N alone are more strongly agglutinated, as a rule, by anti N testing fluids than bloods containing M and N together This observation has been confirmed repeatedly On the average type N blood is twice as sensitive as type MN blood to agglutination with anti N sera²³ This phenomenon has a genetic explanation, type N being homozygous, and type MN heterozygous (cf Chapter XIV)

Although a similar difference in sensitivity exists between type M and type MN bloods with respect to factor M, it is less pronounced than with the factor N

These observations emphasize the importance of including control bloods of all three types in each experiment Particularly when testing for N, blood of type MN (reacting weakly with anti N fluids) must be included as a control

The Agglutinin N₂ The discovery that more than one variety of the property N exists in human blood was the result of the application of the MN tests in cases of disputed paternity (cf page 389) In a paternity case examined by Crome²⁴ the mother's blood seemed to belong to type M, the infant's to type N, apparently contradicting the second law of heredity of the M-N properties (cf page 232) The mother's blood was submitted to a number of independent workers for examination, and three of them (including O Thomsen) obtained weak

²² *Loc cit*

²³ Wiener Zinsher and Sellowe *Jour Immunol* 27 431 (1934)

²⁴ *Deutsche Ztschr f d ges gerichtl Med* 24 167 (1935)

agglutination reactions with the anti N sera they had available Crome therefore concluded this case did not actually contradict the heredity theory since the mother's blood contained a weak N receptor

In an identical case encountered by Friedenreich²⁵ again the mother's blood proved to contain a weakly reacting N receptor As shown in table 41, the special property is detected by only a small proportion of anti N sera Since a study of relatives of the individual in question (cf page 244) established the hereditary nature of this weak reactivity of the blood, Friedenreich concluded that the peculiarity was due to the presence of a special variety of N agglutinogen, design

TABLE 41
REACTIONS OF MN BLOOD WITH VARIOUS ANTI N FLUIDS
(After Friedenreich)

Blood Cells	Anti N Serum No									
	1	2	3	4	5	6	7	8	9	10
MN ₁	-	-	-	(+)	+	+	+(+)	+	-	+(+)
MN	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
M	-	-	-	-	-	-	-	-	-	-

nated by him as N₂, the common N agglutinogen being designated as N₁, in analogy to the varieties of A agglutinogen In contrast to A₂ however, N₂ is extremely rare, comparable in its frequency with the rare property A₂ The present author has encountered only a single blood of type MN₂ among many thousands tested this blood also being detected in connection with a paternity proceeding At the initial tests the putative father's blood was diagnosed as type N, the mother's as type M and the child's as type M, but at the reexamination the child's blood proved actually to belong to type MN₂, justifying the mother's surprise and protestations at the time of the preliminary report Additional examples of blood MN₂ have been reported by Langenberg²⁶ Lauer²⁷ and Pietrusky²⁸

The practical importance of the N₂ agglutinogen is that on account of its weak reactions, it is apt to be missed unless sufficiently potent anti N sera are used

Variations in the M Agglutinin The present author has tested a series of more than 1000 different blood specimens using three different anti M fluids without finding a single discrepancy among the reactions On the other hand Friedenreich

²⁵ *Deutsche Ztschr f d ges gerichtl Med* 25 308 (1936)

²⁶ *Ztschr f Immunitats* 97 48 (1939)

²⁷ *Ztschr f Immunitats* 98 387 (1940)

²⁸ *Ztschr f Immunitats* 99 232 (1941)

and Lauridsen²⁹ found a child of type MN whose blood reacted with only 2 of the 3 anti M sera that he used. Further tests on this blood made with 30 different anti M sera revealed that approximately half of the sera reacted more weakly with the child's blood (5 were quite negative) than with normal MN, one fourth reacted about equally with both, and one fourth of the sera reacted more intensely with the blood of the child than with typical MN. Therefore the M agglutinin in the blood of this child was not merely defective but specialized. The abnormal human M character found by Friedenreich and Lauridsen has nothing to do with the monkey M (cf. page 343) since these authors found that an M serum which reacted with monkey bloods failed to agglutinate the child's blood. The special M agglutinin is evidently extremely rare since another similar blood was not found among 10 000 blood specimens.

SOURCES OF ERROR IN TECHNIC

False Negative Reactions As has already been mentioned, weak immune sera do not yield sufficiently potent testing fluids.

Landsteiner and Levine³⁰ state that they found some anti M sera whose specificity could be recognized by different degrees of agglutination when the exhausted sera were tested against M+ and M- bloods but these sera could not be used for typing because the species agglutinins could not be removed without simultaneous loss of specific action.

The difficulty of the proper preparation of good anti N testing fluids has already been discussed. The main danger here is overabsorption, resulting in testing fluids giving false negative reactions. That these errors are possible is illustrated by the work of Schockaert,³¹ in which this author reported 11 per cent (28 individuals) blood of type M- N- among 245 individuals. On the other hand, other workers who examined more than 100 000 specimens of blood have failed to find a single M- N- blood. In a written communication to the present author, Schockaert attributes his results to the use of too weak testing fluids.

False Positive Reactions Interfering reactions caused by specific agglutinins other than those directed against M and N are not infrequently encountered. Thus, one of the author's immune sera against N, prepared by injecting blood of type ON, were found to contain an immune agglutinin against group O cells, the agglutinin α_2 (cf. page 202). Similar interfering reactions may be caused by natural or immune agglutinins against A and B, the coexistence in the same serum of more than one of the agglutinins against M and N, or possibly by other agglutinins not yet completely analyzed (cf. Chapters XV and XVI). These additional antibodies can be removed by suitable absorption procedures. E.g. when preparing the anti N testing fluid, if the diluted

²⁹ Friedenreich and Lauridsen *Acta Path et Microbiol Scand* Supp. 38: 155 (1938).

³⁰ *Loc cit*.

³¹ *Compt rend Soc Biol* 103: 544 (1930).

immune serum is absorbed with a mixture of bloods of types OM, AM, and BM, the danger of such extraneous reactions will be practically eliminated

A more frequent cause for false positive reactions is under absorption of the immune sera. This danger, like that of overabsorption applies mainly to the tests for N. This error is prevented by the inclusion of negative control bloods. When the tests for N are performed by less reliable technics, e.g., on the open slide or by the hanging drop errors are more apt to occur.

In this connection attention should be called to some calculations made by Koller³² which show that in cases of disputed paternity in which the properties M and N are applied there is a greater possibility of error as a result of over absorption than from incomplete absorption.

DEVELOPMENT OF THE AGGLUTINOGENS M AND N

The sensitivity of the M and N agglutinogens in the blood of new born is equal to that of adult blood, judging from the results of titration experiments with anti M and anti N sera.³³ This indicates that the M N properties are fully developed at birth.³⁴ Moreover Hyman³⁵ observed no changes in the M N types of 38 infants studied following birth for periods up to 18 months. Moureau³⁶ has been able to demonstrate the presence of the M N properties in the blood of human fetuses as early as the second month of intra uterine life and Hyman states that the type is fixed before the seventh month. These observations are of significance in relation to the medicolegal applications of the M and N factors.

³² *Ztschr f Rassenphysiol* 5 102 (1932)

³³ Wiener unpublished observations

³⁴ Schiff [*Deutsch Ztschr f d ges gerichtl Med* 18 4* (1931)] arrived at the same conclusion because he observed that the distribution of the M N types in newborn and adults was the same. Moreover there were no contradictions to the heredity theory as should be the case if the M N types were not completely developed at birth.

³⁵ *Jour Immunol* 43 1 (1942)

³⁶ *Rev belge des Sciences Med* Vol VII (1935)

CHAPTER XIV

HEREDITY OF THE AGGLUTINOGENS M AND N

THE FIRST report on the heredity of the agglutinogens M and N was made by Landsteiner and Levine.¹ As a result of a study of 64 families with 286 children, these authors succeeded in demonstrating that the agglutinogens, M and N, are inherited as Mendelian dominants, and that their heredity probably depends upon a single pair of allelic genes, *M* and *N*. According to this theory, only three genotypes are possible, *MM*, *MN*, and *NN*, corresponding to the three phenotypes, M, MN, and N, respectively. The theory therefore accounts for the nonexistence of type M-N—

TABLE 42
HEREDITY OF THE AGGLUTINOGENS M AND N

Types of Parents	Per Cent Children of Types		
	MN	M	N
MN × MN	50	25	25
MN × N	50	0	50
MN × M	50	50	0
M × N	100	0	0
M × M	0	100	0
N × N	0	0	100

Six different matings are possible. It is a simple matter to determine what the types of the children must be when the types of the parents are known. For example, in the mating $MN \times M$, the genotype of the former parent must be *MN*, so that the parent will produce equal numbers of germ cells bearing gene *M* and gene *N*, whereas the latter parent, of genotype *MM*, can only produce germ cells bearing gene *M*. By random fertilization two kinds of zygotes, *MM* and *MN*, will be produced in equal numbers, so that half of the children must belong to type MN, and half to type M. The other five matings can be analyzed in a similar manner (cf. table 42).

According to the theory of Landsteiner and Levine, the following two rules should hold:

1. Agglutininogen M cannot appear in the blood of a child unless pres-

¹ *Jour. Exp. Med.* 48: 731 (1928).

ent in the blood of one or both parents, the same rule holds for agglutinin N

2 A type M parent cannot give rise to a type N child, and conversely, a type N parent cannot give rise to a type M child. This follows from the fact that a type M individual is of genotype *MM*, and consequently can only produce germ cells bearing the gene *M*. Each child must therefore possess at least one *M* gene, and cannot belong to

TABLE 43
HEREDITY OF THE M N TYPES IN 286 FAMILIES

Parental Combination	Number of Families		Children belonging to type			
			M	N	MN	Totals
M × M	24	{ Number Per Cent	98 99.0 ± 0.7	0 0	(1) 1.0 ± 0.7	99
M × N	30	{ Number Per Cent	0 0	0 0	113 100	113
N × N	6	{ Number Per Cent	0 0	27 100	0 0	27
MN × M	86	{ Number Per Cent	183 48.3 ± 1.7	0 0	196 51.7 ± 1.7	379
MN × N	71	{ Number Per Cent	(1) 0.3 ± 0.3	167 51.5 ± 1.8	156 48.2 ± 1.8	324
MN × MN	69	{ Number Per Cent	71 25.8 ± 1.7	63 22.9 ± 1.7	141 51.3 ± 2.0	275
Totals	286		353	257	607	1217

This table includes

131 families with 642 children reported by Wiener and Vaisberg [*Jour Immunol* 20: 371 (1931)]

56 families with 205 children by Zieve, Wiener and Fries [*Ann Eng* 7: 163 (1936)]

59 families with 232 children by Landsteiner and Wiener [*Jour Exp Med* 74: 309 (1941)]

40 families with 138 children by Wiener and Sonn [*Genetics* March (1943)]

genotype *NN* (type N). Similar reasoning holds for the reverse combination.

In table 43 are presented the author's investigations on the heredity of the M N types, comprising 286 families with 1,217 children. The distribution of the types among the children of each of the six sorts

of matings conforms closely with that expected according to the theory of Landsteiner and Levine, the deviations in no case reaching twice the probable error. There were two contradictions to the rules of heredity: (1) a type MN child in a family where both parents belonged to type M and (2) a type M child in a mating $MN \times N$. In both these cases, however, there is reason to doubt the legitimacy of the child.

In table 44 are summarized all the studies on the heredity of the agglutinogens M and N that have been published to date, including the data from table 43. Among 2165 families with 6718 children there were 3 contradictions to the first law of heredity and 10 to the second law. These apparent exceptions can all be attributed to illegitimacy, however. This is true for the 10 "exceptions" to the second law, since in every case the parent involved was the father. If the theory were at fault, exceptions should have been found concerning the mother. To date more than 14,601 mother-child combinations have been examined (cf. table 45), and not once have the combinations, type M mother with type N child or type N mother type M child, been encountered. Since it can be shown by calculation² that for every two apparent exceptions to the second rule which can be explained by illegitimacy there is one exception to the first law, the three exceptions to the first law in table 44 can also reasonably be attributed to illegitimacy.

It has been calculated that where a man has been falsely accused of paternity, the chances of disproving the charge by means of the tests for M and N are approximately equal to the chances using the classic blood groups (cf. page 387). If the assumption that the "exceptions" listed in table 44 are all due to illegitimacy is correct, then the incidence of such exceptions to the heredity theory of Landsteiner and Levine should be about the same as the frequency of exceptions to the theory of heredity of the blood groups. As a matter of fact, as shown in table 44, among 6718 children, 13 contradictions to the theory of Landsteiner and Levine were found, and in table 15, among 24,343 children, 58 discrepancies with Bernstein's theory are listed. This supports the view that the exceptions observed are due to illegitimacy.

In this connection, Crome's³ experience may be cited. In one of the families that he studied, the examination of the bloods of parents and children yielded the following results: Father, AN, Mother, AN, Children 1 AN, 2 AMN, 3 ABMN, 4 ABMN, 5 BBMN. Children 2-5 therefore possess agglutinogen M which is absent from the bloods of both parents, and children 3-5 possess agglutinogen B which is also absent from the parents' bloods. From other evidence, it could be demonstrated that these children were all illegitimate.

Another pertinent observation is that of the 13 contradictions to the heredity theory (cf. table 44) 5 are derived from the original study of Landsteiner and Levine on only 286 children. As was pointed out by these investigators, the families studied by them were taken from strata of society where the environment favors

² Wiener, *Jour. Immunol.* 19: 259 (1930), *ibid.* 24: 443 (1933).

³ *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 20: 316 (1933).

TABLE 41

SUMMARY OF PUBLISHED DATA ON THE HEREDITY OF THE M \ N TYPES

Parental Combination	Number of Families	Number of Children of Type			
		M	N	MN	Totals
M × M	205	594	0	(3)	597
M × N	226	(1)	(2)	698	701
N × N	104	0	323	0	323
MN × M	588	860	(5)	988	1853
MN × N	426	(2)	682	688	1372
MN × MN	616	417	361	1094	1872
Totals	2165	1874	1373	3471	6718

This table includes

- 64 families with 286 children by Landsteiner and Levine [*Jour Exp Med* 48 731 (1928)]
- 108 families with 293 children by Schiff [*Deutsch Ztschr f d ges gerichtl Med* 21: 404 (1933)]
- 390 families with 577 children by Clausen [*Hospitalstud* 75 196 (1932)], of which 102 families with 103 families had previously been reported by Thomsen and Clausen [*Hereditas* 15 213 (1931)]
- 117 families with 202 children by Lattes and Garrasi [*Atti del II Congresso Naz di Microbiol*, p 146 (1932)]
- 80 families with 280 children by Blaurock [*Munch med Woch* 79 1552 (1932)]
- 200 families with 730 children by Moureau [*Revue belge des Sciences Méd* 7 155, 540, 589 (1935)]
- 100 families with 220 children by Hyman [*Jour Immunol* 29 223 (1935)]
- 103 families with 210 children by Furuhata, Imamura and Sugishita [*Hanzai-gaku Zasshi* 9 54 (1935)]
- 29 families with 72 children by Mustakallio [*Acta Soc Med Fenn Duodecim* 20 No 2 (1937)]
- 113 families with 315 children by Matta [Publication No 11, Faculty of Medicine of the Egyptian University (1937)]
- 121 families with 528 children by Okaya [*Hanzai-gaku Zasshi* (Japanese) 12 589 (1938)]
- 35 families with 67 children by Hirszfeld and Kostuch [*Schweiz Ztschr f Allgem Path u Bakter* 1 23 (1938)]
- 109 families with 480 children by Dahr and Bussman [*Deutsch med Woch* 64 818 (1938)]
- 107 families with 249 children by Taylor and Prior [*Ann Eug* 8 343 (1938)]
- 100 families with 234 children by Holford [*Jour Inf Dis* 63 287 (1938)]
- 112 families with 424 children by Dahr [*Ztschr f Immunitäts* 97 170 (1939)]
- 98 families with 314 children by Dahr, Ofte and Weber [*Ztschr f Rassenphysiol* 11 78 (1940)]
- and 286 families with 1217 children from table 43.

This table does not include Nicoletti's data [*Riv d pat sper* 10 8 (1933)] which were not available for analysis, and the study by Kerkhoff [*Ztschr f Rassenphysiol* 12 28 (1941)]

a high incidence of illegitimacy. In their study accordingly they encountered apparent contradictions not only to the laws of heredity of the M N types but also to the heredity of the blood groups.

In view of the above considerations, and, in particular, the results of the studies on mothers and children, it is evident that *not a single proven exception to the theory of Landsteiner and Levine has been*

TABLE 45
SUMMARY OF REPORTED MOTHER CHILD COMBINATIONS

Authors	Number of Mothers	Number of Children
Families listed in table 44	2165	6718
Crome* (families)	22	50
Mayser† (families)	65	209
Schiff‡ (newborn)	660	662
Schiff‡ (forensic cases and others)	1905	1927
Wiener, Rotbberg and Fox§	461	497
Wiener (forensic cases)	489	509
Mayser†	85	85
Crome*	486	498
Moureaux (newborn)	461	461
Meixner¶ (newborn)	40	40
Wolff and Jonsson** (forensic cases)	709	732
Laves††	278	279
Puschel‡‡ (forensic cases)	156	156
Friedenreich§§ (forensic cases)	1778	1778
Totals	9760	14601

* *Deutsche Ztschr f d ges gerichtl Med* 20 316 (1933) *ibid* 21 435 (1933)

† *Aerzt Sachverst Zeitung* 38 198 (1932)

‡ *Deutsche Ztschr f d ges gerichtl Med* 21 404 (1933)

§ *Jour Immunol* 23 63 (1932)

|| Unpublished

¶ *Wien klin Woch* 79 1552 (1932)

** *Acta Pathol et Microbiol Scand* 12 131 (1935)

†† *Festschr Ver Aerzt Steiermark* p. 50 (1933)

‡‡ *Ztschr f Immunitats* 81 445 (1934)

§§ Cited after Thomas [*Jour Crim Law* 1 598 (1937)]

encountered to date in studies on a total of more than 15,000 children. Accordingly, the M N tests have been applied in medicolegal cases for the exclusion of parentage, and this has resulted in a further confirmation of the theory. As has already been mentioned, in a few such forensic exceptions were encountered, but these were explained by the demonstration of a special type of blood, MN₂ (cf page 227). Thus,

TABLE 46

ANALYSIS OF MATINGS $MN \times MN$ FROM REPORTED STUDIES ON THE HEREDITY OF THE MN TYPES

Investigators	Number of Families	Types of Children				Per Cent Type MN Children
		M	N	MN	Totals	
Landsteiner & Levine	11	17	7	31	55	56.4 ± 4.5
Wiener & Vaisberg	25	23	29	58	116	50.0 ± 3.1
Schiff	33	18	22	48	88	54.5 ± 3.7
Clausen	70	38	28	74	140	52.8 ± 2.8
Lattes & Garrasi	54	16	17	65	98	66.3 ± 3.2
Blaurock	23	25	25	40	90	44.4 ± 3.8
Moureaux	53	45	41	102	188	54.2 ± 2.4
Hjman	32	10	16	41	67	61.2 ± 3.0
Furubata Imamura Sugushita	32	7	20	30	57	54.4 ± 4.4
Zieve Wiener Fries	18	12	17	28	57	49.1 ± 4.4
Mustakallio	4	2	4	10	16	62.5 ± 8.1
Matta	20	9	10	45	64	70.3 ± 4.4
Okaya	21	26	22	74	122	60.7 ± 3.0
Hirszfeld & Kostuch	17	7	5	21	33	63.6 ± 5.6
Dahr & Bussman	30	38	18	70	126	55.5 ± 2.3
Taylor & Prior	23	10	8	38	56	67.5 ± 4.2
Dahr	57	29	24	165	218	75.7 ± 1.9
Dahr Offe & Weber	33	25	17	67	104	59.6 ± 3.2
Holford	34	24	14	37	75	49.3 ± 3.9
Landsteiner & Wiener	19	22	13	43	78	55.1 ± 3.7
Wiener (unpublished)	7	8	4	12	24	50.0 ± 6.7
Totals	616	417	361	1094	1872	58.4 ± 0.7

the application of the theory of Landsteiner and Levine was responsible for the discovery of the existence of serological variations in the N agglutinin

When the distribution of the MN types among the children of the 2165 families summarized in table 44 is compared with that expected under the theory of Landsteiner and Levine it is seen that a few of the frequencies deviate significantly from the expected values. This is particularly noticeable in the mating $MN \times MN$ which has a large excess of type MN children. Taylor and Prior* have been greatly impressed by this result. However they propose no explanation for the observation although they do not believe it can be ascribed to imperfections in technique. As a matter of fact the peculiar observations in the mating $MN \times MN$ are comparable to those reported in the mating $A \times B$ (cf. page 191). In these two matings children of all types and groups are possible so that the investigators had a tendency to focus less attention on such families. In the other matings errors in technique are apt to lead to contradictions to the heredity laws and this serves to call the attention of the investigator to the mistake. That this explanation is correct becomes evident if the $MN \times MN$ matings are analyzed according to the investigators (cf. table 46).

*Ann. Eug. 9:18 (1939)

While, as shown in table 46 in the combined studies of all workers there were 58.4 ± 0.7 per cent children of type MN the frequencies obtained by the individual investigators differed markedly, ranging from 44.4 to 75.7 per cent. The only plausible explanation for this lack of homogeneity of the results is the differences in the accuracy of the tests when conducted by different workers. As has already been pointed out, the frequency observed by the present author was 51.3 ± 2.0 , which does not differ significantly from 50 per cent. On the other hand, the results obtained by Dahr (75.7 ± 1.9) and Lattes and Garrasi (66.3 ± 3.2) may be attributed to the use of incompletely absorbed testing fluids. With regard to the results of the latter investigators see page 239. Dahr's results differ markedly not only from the expected values but also from those previously reported by Dahr himself in a study carried out in collaboration with Bussman. It seems reasonable to assume that the bias due to the use of insufficiently absorbed testing fluids would tend to be the same in direction in the majority of the reports though varying in degree.

Theoretical and Statistical Considerations If a given population is homogeneous with respect to M and N and if the frequencies of the three types M, N and MN, in the population are known it is possible to calculate the frequencies of the genes, \bar{M} and \bar{N} , in the population. In heterogeneous populations after a single generation of random intermarriage equilibrium will be reached* since only a single pair of allelic genes is involved (cf. page 176). If m represents the frequency of germ cells bearing gene M , and n the frequency of germ cells bearing gene N , then $m + n = 1$ by definition. If \bar{M} , \bar{N} , and \bar{MN} denote the frequencies of the three types, then

$$m = \bar{M} + \frac{\bar{MN}}{2} \quad (1)$$

$$n = \bar{N} + \frac{\bar{MN}}{2} \quad (2)$$

For the frequency of each gene is necessarily equal to the frequency of the corresponding homozygous type plus half the frequency of the heterozygous type.

Furthermore the frequencies of the phenotypes can be expressed in terms of the frequencies of the genes as follows:

Phenotype	Genotype	Frequency	
\bar{M}	MM	m^2	(3)
\bar{MN}	MN	$2mn$	(4)
\bar{N}	NN	n^2	(5)

$$\bar{M} + \bar{MN} + \bar{N} = m^2 + 2mn + n^2 = (m + n)^2 = 1$$

$$\text{From (3), } m = \sqrt{\bar{M}} \quad (6)$$

$$\text{and from (5), } n = \sqrt{\bar{N}} \quad (7)$$

$$\text{But } m + n = 1$$

Therefore, if the theory of Landsteiner and Levine is correct, the following relationship between the frequencies of the types must hold

$$\sqrt{\bar{M}} + \sqrt{\bar{N}} = 1 \quad (8)$$

*Wiener Jour Immunol 21: 157 (1931)

TABLE 47
STATISTICAL TEST OF THE THEORY OF LANDSTEINER AND LEVINE

Authors	Population	Frequency of Types			Num ber of Indi viduals	$m = \sqrt{M}$	$n = \sqrt{N}$	$m \pm n$	D	P.E.
		MN	MI	N						
Landsteiner and Levine	New York City	53.6 \pm 1.50	26.1	20.3	532	51.12	45.02	96.18	-3.82	1.46
	a Whites	47.5	27.6	24.9	181	52.56	49.90	102.46	+2.46	2.51
	b Colored									
	American Indians									
Wiener and Vaisberg	a Tallahassee, Kans	33.33	62.97	3.70	81	79.35	19.24	98.56	-1.41	3.75
	b Lawrence, Kans	36.29	58.07	5.64	124	76.20	23.76	99.96	-0.04	3.03
	Brooklyn, N.Y. (Families)	48.23	30.53	21.24	904	55.25	46.09	101.34	+1.34	1.12
	Brooklyn, N.Y.									
Wiener, Rothberg, and Fox	a Mothers	50.32 \pm 1.57	28.63	21.05	461	53.51	45.88	99.39	-0.61	1.57
	b Children	50.71 \pm 1.51	29.98	19.31	497	54.75	43.94	98.69	-1.31	1.51
	Berlin									
Schuff	a First series	49.01	30.14	20.85	1420	54.89	45.66	100.55	+0.55	0.89
	b Second series	49.77	31.52	18.71	1913	56.14	43.26	99.40	-0.60	0.77
	Total	49.44	30.94	19.62	3333	55.62	44.30	99.92	-0.08	0.58
	Volgadeutsche	48.89	23.38	27.78	180	48.31	52.71	101.02	+1.02	2.51
Shigeno	Japanese									
	a First series	46.91	29.78	23.41	141	54.57	48.37	102.94	+2.94	2.84
	b Second series	44.26	31.15	24.59	61	53.81	49.58	103.38	+5.39	4.31
	Total	45.80	30.30	23.90	202	55.05	48.89	103.94	+3.94	2.37
Blairlock	Cologne	49.1	29.1	21.5	2000	53.94	46.37	100.31	+0.31	0.75
	Copenhagen	44.57	29.86	25.57	412	54.64	50.56	105.20	+5.20	1.60
	Italians	57.4 \pm 1.61	27.2	15.3	480	52.15	39.12	91.27	-8.73	1.63
	French	45.80	33.00	21.20	400	57.45	46.04	103.49	+3.49	1.68
Kosovitch	Germans	49.0	32.5	18.5	1300	57.01	43.01	100.02	+0.02	0.94
	Frankfurt a M	52.9 \pm 1.06	27.0	20.1	1000	52.06	44.83	96.82	-3.11	1.07

In practice of course the relation will not hold exactly (cf page 171) As Wiener⁶ has shown if the deviation

$$D = 1 - (\sqrt{M} + \sqrt{N})$$

$$\text{Then P E D (probable error of D)} = \frac{0.6745}{2\sqrt{V}} \quad (9)$$

where V represents the number of individuals in the sample of the population examined

It is possible therefore to test the theory of Landsteiner and Levine by determining the frequencies of the three types in various homogeneous populations and then calculating whether or not equation (8) holds satisfactorily (by comparing D with its probable error) In table 47 are summarized the results of such calculations for the populations studied up to the year 1932 It may be seen that in 11 of the 18 independent studies D is less than the probable error which is very satisfactory since the probability theory requires that the deviation be less than the probable error in only half of the cases The excellence of this fit is all the more remarkable since several of the populations are not homogeneous (eg the populations from Brooklyn and New York City) and some of the samples are not random but consist of groups of families In one case however the deviation is more than 5 times the probable error (Lattes and Garrasi) A deviation greater than 5 P E occurs only once in 1300 trials (cf page 170) so that the likelihood of this being a purely accidental deviation is small Perhaps the fluids used were insufficiently absorbed so that a number of false positive reactions was obtained (cf page 237)

Dr R A Fisher of the Rothamsted Experimental Station has suggested the application of the χ^2 test to the theory of Landsteiner and Levine If a b and c represent the number of individuals of types M MN and N respectively in a sample of population containing V individuals (so that $a + b + c = V$) the maximum likelihood estimates of the frequencies of the genes are ⁷

$$m = \frac{2a+b}{2V} \quad (10)$$

$$n = \frac{2c+b}{2V} \quad (11)$$

(These formulae are the same as formulae (1) and (2) except that absolute values are used here instead of percentage frequencies)

In the following equations the letter with the subscript o represent theoretically expected frequencies the letters without subscripts those actually observed

$$\chi^2 = \sum \frac{(x - x_o)^2}{x_o} = \frac{(a - a_o)^2}{a_o} + \frac{(b - b_o)^2}{b_o} + \frac{(c - c_o)^2}{c_o}$$

$$\text{Since } a_o = V m^2 = V \left(\frac{2a+b}{2V} \right)^2$$

$$\text{And } b_o = V 2mn \\ c_o = V n^2$$

$$\text{Therefore } \chi^2 = \frac{(b^2 - 4ac)V}{(2a+b)^2(b+2c)^2} \quad (12)$$

⁶ *Jour Immunol* 21 157 (1931)

⁷ For the proof of this statement and also for the detailed derivation of the various formulae given in this section see Wiener *Human Biology* 7 222 (1935)

⁸ This formula was first derived by Fisher (personal communication)

TABLE 48

 χ^2 -TEST OF THE THEORY OF LANDSTEINER AND LEVINE

Investigator	Frequency of Type			Number of Individuals Examined	χ^2	P
	MN	M	N			
<i>Landsteiner Levine</i>						
Whites (New York)	53.6	26.1	20.3	532	3.04	0.09
Colored	47.5	27.6	24.9	181	0.35	0.55
American Indians	35.12	60.00	4.88	205	0.021	0.89
<i>Wiener Vaisberg</i>						
Families (Brooklyn, N.Y.)	48.23	30.53	21.24	904	0.66	0.42
<i>Wiener Rothberg Fox</i>						
Mothers	50.32	28.63	21.05	461	0.069	0.79
Children	50.71	29.98	19.31	497	1.06	0.30
<i>Schiff</i>						
Berlin	49.44	30.94	19.62	3333	0.0089	0.92
Wolgadeutsche	48.89	23.38	27.78	180	0.078	0.78
<i>Shigeno</i>						
Japanese	45.80	30.30	23.90	202	1.30	0.25
<i>Blaurock</i>						
Cologne	49.1	29.1	21.5	2000	0.17	0.68
<i>Thomsen Clausen</i>						
Copenhagen	44.57	29.86	25.57	442	1.36	0.24
<i>Lattes-Garrasi</i>						
Italians	57.4	27.2	15.4	430	12.17	<0.001
<i>Kossoritch</i>						
French	45.80	33.00	21.20	400	2.17	0.14
<i>Laubenheimer</i>						
Frankfurt a. M.	52.9	27.0	20.1	1000	4.10	0.05

(The formula for χ^2 is the same whether absolute values or percentage frequencies are used.) The value of χ^2 , in general, depends on the closeness of fit between the observed and theoretical values and the number of independent classes (or degrees of freedom). In Fisher's book there are tables with values for P which represents the probability, by chance alone, of exceeding a given value of χ^2 for a given number of degrees of freedom. In formula (12) above, the number of degrees of freedom is taken equal to one.

In table 48, the statistics given in table 47 are analyzed by the χ^2 method. The agreement between observation and expectation is good except in one case.

It has already been pointed out that $\overline{MN} = 2mn$. Since $m + n = 1$, type MN will have its maximum frequency when $m = n = \frac{1}{2}$. The maximum possible frequency of type MN therefore is $2(\frac{1}{2})(\frac{1}{2}) = \frac{1}{2}$ or 50 per cent. In 5 populations listed in table 47, the frequency of type MN is greater than 50 per cent. In 4 of these 5 populations, however, the difference from 50 per cent is not significant when compared with the probable error. (The formula for the probable error of a frequency is given on page 170.) In the fifth case, the expectation is not fulfilled, as with the other calculations.

A further statistical test of the theory of Landsteiner and Levine can be made by a study of the agglutinogens M and N in mothers and children (Wiener et al.⁹) Formulae for the frequencies of the various mother child combinations in terms of the frequencies of the genes are given in table 49 To illustrate the method of derivation of these formulae let us consider the combination, type M mother with type M child This combination can arise only from the following two matings

M mother \times M father
M mother \times MN father

The former mating has the frequency $m^2 \times m^2 = m^4$, and the latter mating has the frequency $m^2 \times 2mn = 2m^3n$ Since all the children in the former mating and half

TABLE 49
FREQUENCIES OF MOTHER CHILD COMBINATIONS

Mothers of Type	Children of Type		
	M	MN	N
M	m^4	m^3n	0
MN	m^3n	mn	mn^2
N	0	mn^2	n^4

the children in the latter mating are of type M the desired frequency is $m^4 + m^3n = m^3(m+n) = m^3$

Application of the formulae to actual observations has resulted in complete agreement with the expectancies.¹⁰ The values of m and n to be used in the formulae are derived by means of equations (1) and (2) given above

As a result therefore of all studies made up to the present time the theory of Landsteiner and Levine that the heredity of the agglutinogens M and N depends upon a single pair of allelic genes neither of which is recessive is well established The evidence in favor of the theory may be classified as follows

1 Observations on Families

a Qualitative (Non-existence of true exceptions to the two laws of heredity)

b Quantitative (Cf table 43)

2 Observations on Mothers and Children

a Qualitative (Non-existence of a single combination of type M mother with type N child or vice versa among more than 15 000 pairs studied to date)

b Quantitative (Cf table 49)

3 Analysis of Statistics in Populations

a Qualitative (Non existence of the type M N among more than 100 000 specimens of blood studied up to the present time)

b Quantitative (Cf table 47)

For certain calculations it is desirable to have formulae for the frequencies of

⁹ Jour Immunol 19 259 (1930)

¹⁰ Schuff Klin Woch 8 448 (1929), *ibid* 9 1956 (1930), Wiener Rothberg and Fox Jour Immunol 23 63 (1932)

the genes that add up to unity, and at the same time have minimum probable errors. It has been shown that equations (1) and (2) satisfy both these requirements.¹¹

Constancy of the Types It is to be expected that the type of an individual with respect to the agglutinogens M and N will remain constant throughout life. As a matter of fact, although the bloods of members of our laboratory staff have been repeatedly examined for the agglutinogens M and N for a period of more than twelve years, in no case has a change in type been noted. This same observation has been made by others. This constancy of the three blood types of Landsteiner and Levine makes feasible their medicolegal application.

A temporary, apparent change in blood type has been repeatedly observed following blood transfusion. Since the agglutinogens M and N need not be taken into account when selecting donors for transfusions, the patient will often receive blood that differs in agglutinin content (with respect to M and N) from his own blood. The transfused blood may remain in circulation for periods up to three or four months after the transfusion (cf. page 73), and this may cause mistakes when the patient's blood is typed during such an interim. Though such circumstances will rarely obtain in medicolegal cases, the possibility should be borne in mind.¹² However, if transfused cells persist in the circulation, the microscopic picture will be different from the ordinary positive tests.

The Agglutinogens M and N and Sex It has been found that the distribution of the agglutinogens M and N is independent of sex, since the frequencies of the three types is the same in the two sexes. Furthermore, family studies reveal that these agglutinogens are not sex-linked, so that the genes, M and N, are not located in the sex chromosomes.

Relationship of the Agglutinogens M and N to the Agglutinogens A and B Landsteiner and Levine pointed out that the distribution of the agglutinogens M and N is not different in the four blood groups, O, A, B, AB, so that no correlation exists between the factors M and N and the factors A and B. In addition, from an inspection of their data on the simultaneous heredity of the four agglutinogens, A, B, M, and N, Landsteiner and Levine concluded that the agglutinogens M and N are probably inherited independently of the agglutinogens A and B. This conclusion has been confirmed by mathematical calculations made independently by Bernstein¹³ and by the writer.¹⁴

Whereas the determination of the presence or absence of sex linkage is a simple matter, it is difficult to detect somatic linkage in man. From an inspection of family studies, only close linkage would be detected. Until ten years ago no method of detecting loose linkage in data based on only two generations was available. As an example of the principles involved, the methods used for demonstrating the absence

¹¹ Wiener, *Human Biology* 7: 222 (1935)

¹² Such instances have actually been encountered by the author in forensic cases.

¹³ *Ztschr. f. indukt. Abstamm. u. Vererb.* 57: 113 (1931)

¹⁴ Wiener, *Genetics* 17: 335 (1932)

of linkage between the agglutinogens M and N and the agglutinogens A and B will be discussed in some detail

The only type of families in which the presence or absence of linkage can make any difference in the types of the offspring is that in which one of the parents is doubly heterozygous. That is one of the parents must belong to one of the following types $ABMN$, A_hMN and B_hMN (The subscript 'h' stands for the heterozygous genotype. In the families where there are group O children from group A or B parents these parents are necessarily heterozygous.) The offspring of each family may be subdivided into three classes "linked" "cross overs" and 'indeterminate'.¹⁴ Thus, in the cross $ABMN \times OMN$, if the linked genotype of the $ABMN$ parent is assumed to be $(AM)(BN)$ the linked gametes from that parent are (AM) and (BN) and the cross over gametes are (AN) and (BM) . The OMN parent produces (OM) and (ON) gametes in equal numbers. The linked zygotes therefore are $(AM)(OM)$, $(AM)(ON)$, $(BN)(OM)$ and $(BN)(ON)$, and the cross over zygotes are $(AN)(OM)$, $(AN)(ON)$, $(BM)(OM)$ and $(BM)(ON)$. Hence the linked types are AM and BN and the cross over types are AN and BM , whereas the types AMN and BMN might be either linked or cross overs and may therefore be called 'indeterminate'. A table of 39 different matings worked out by the method just described is given in the paper by Wiener.¹⁵

If it could be determined whether an individual of type $ABMN$ belongs to genotype $(AM)(BN)$ or $(AN)(BM)$, and similarly for the types A_hMN and B_hMN the cross-over frequency could be determined directly. This might be done by determining the types of the grandparents. Eg., an individual of type $ABMN$ with parents AM and BMN would belong to genotype $(AM)(BN)$, such an individual with parents AN and BMN would belong to genotype $(AN)(BM)$ but if the parents are AMN and BMN the genotype is indeterminate. Unfortunately however it is very difficult for obvious reasons to obtain three generations in studies on human heredity. It is therefore necessary to devise a method of measuring linkage based on data from only two generations and involving only comparatively small families.

Bernstein solved this problem by taking the product of the two classes linked and cross-overs. He found that this product depends upon the number of children in the family and the cross-over frequency. In this way the necessity of determining the genotype of the doubly heterozygous parent was eliminated. The writer showed that a similar result can be obtained by making summations ΣU and ΣV where U and V represent the larger and smaller respectively of the two classes linked and cross-overs in each family. These sums depend upon the size of the families and the cross over frequency so that the latter could be determined by consulting special tables constructed for the purpose.

If the computed cross over frequency is found to be significantly less than 50 per cent, the presence of linkage is established. On the other hand if the difference from 50 per cent is not significant the presence of linkage becomes very improbable. This is especially true since man possesses 24 pairs of chromosomes so that *a priori*, the likelihood of linkage between two somatic (not sex linked) traits is small (only 1/23). Since Bernstein and Wiener both failed to find any evidence for linkage of M and N to A and B it may be considered established that the genes M and N are not located in the same chromosomes as the genes, A, B, and O.

¹⁴ These terms are used for convenience without commitment as to the presence or absence of linkage.

¹⁵ Cf Wiener, Zieve and Fries *Ann. Eug.* 7, 163 (1936).

Heredity of Agglutinin N_2 . The observation by Friedenreich of an individual of type MN whose blood is characterized by an extremely weak reactivity with anti-N testing fluids has been cited above (cf page 228). When tests were made on other members of this person's family (cf figure 54), 12 other individuals were found whose blood

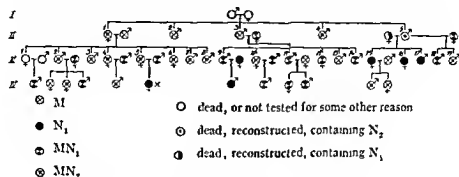


FIG 54 PEDIGREE DEMONSTRATING THE HEREDITARY NATURE OF THE AGGLUTINOGEN N_2
(After Friedenreich)

behaved in the same manner. To account for his findings, Friedenreich has postulated the existence of 3 allelic genes, M , N_1 and N_2 , where gene N_1 determines the usual N agglutinin, and the rare gene N_2 determines the atypical, weakly reacting N agglutinin. A number of small pedigrees illustrating the hereditary transmission of the MN_2 type have also been reported by Pietrusky.¹⁴

¹⁴ *Ztschr f Immunitäts* 98 387 (1940)

CHAPTER XV

FACTORS RH AND ANTI RH OF HUMAN BLOOD*

WITH THE agglutinogens A₁, A₂, B, M and N described in the previous chapters, eighteen types of human blood are readily distinguished. Attempts to discover additional agglutinogens by the familiar technic used for demonstrating factors M and N, namely, by immunizing rabbits with human blood, have yielded few positive results (cf page 264). Approaching the problem in another way, Landsteiner and Wiener¹ immunized rabbits with animal instead of human blood, considering that some animal bloods contained antigens related to agglutinogens in individual blood. For example the Forssman substance in sheep cells is related to agglutininogen A of human blood (cf page 31). One result which favored this plan had been previously obtained by these workers,² namely, that certain anti rhesus sera contained agglutinins specific for the human agglutininogen M (cf page 343). Continuing this line of investigation, by immunizing rabbits with rhesus blood, Landsteiner and Wiener obtained an immune serum with which an agglutinable factor in human blood different from A, B, M, N or P was determined, and this new factor was designated as Rh, to indicate the use of rhesus blood in producing the serum. The property Rh was found to be present in the blood of about 85 per cent of white individuals, and independent of the blood group and M N type. Shortly after the property Rh was discovered, Wiener and Peters³ demonstrated its importance as a cause of hemolytic transfusion reactions, as has already been pointed out (cf page 65). Moreover, Levine, Katzin and Burnham⁴ found that this factor plays an important role in the pathogenesis of erythroblastosis fetalis (cf page 369).

In view of the clinical importance of the Rh factor detailed directions as to the technic of testing for the property are given here.

Testing Sera. While in their original study Landsteiner and Wiener obtained the immune serum by immunizing rabbits they subsequently found that such sera are far more easily produced in guinea pigs.⁵ Hu

* The author's investigations on the Rh factor were aided by grants from the Committee on Human Heredity of the National Research Council and the Committee on Scientific Research of the American Medical Association.

¹ *Proc Soc Exp Biol and Med* 43 223 (1940)

² Landsteiner and Wiener *Jour Immunol* 33 19 (1937)

³ *Ann Int Med* 13 2306 (1940)

⁴ *Jour Amer Med Assoc* 116 825 (1941)

⁵ *Jour Exp Med* 74 309 (1941)

man sera containing anti-Rh agglutinins may be obtained from certain patients who have had hemolytic transfusion reactions following transfusions of blood of the homologous group⁸ and often from mothers of infants with the disease erythroblastosis fetalis.⁷

For the preparation of the immune guinea pig sera the following technic has been used with good results by Landsteiner and Wiener. Large guinea pigs are injected intraperitoneally with a suspension of washed rhesus blood cells, each animal being given a dose equivalent to 2 c.c. of whole blood. The injections are repeated after five days, and one week later the animals are bled. While the sera of the majority of the guinea pigs will be found to show differences between the two sorts of blood, Rh positive and Rh negative, only one or two sera out of every ten will prove suitable for practical diagnosis. The simplest way to select satisfactory sera is to prepare a series of progressively doubled dilutions of each serum and test these against suspensions of Rh negative and Rh positive blood. Those sera which give in three successive dilutions negative reactions with the former but positive with the latter type of blood are suitable. Then all that is required is to select a dilution of the serum, for example, 1:10, which gives no reaction with negative blood but definite reactions with positive blood. With occasional sera containing appreciable amounts of anti A and anti B agglutinins it may be necessary to absorb with small amounts of group A and group B bloods. The guinea pig sera need not as a rule be inactivated since they are diluted for the actual tests.

More recently Landsteiner and Wiener⁸ have modified their technic by continuing the immunization longer so that each guinea pig receives 5 instead of only 2 injections. The proportion of usable sera is higher by this method. Satisfactory sera are pooled and preserved with merthiolate 1:5000. Probably because of the higher antibody content of such sera, simple dilution is not adequate and absorption is essential in order to obtain a reagent distinguishing sharply between positive and negative bloods.

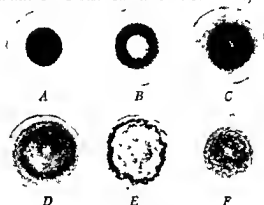
To determine whether human sera from patients with hemolytic transfusion reactions or from mothers with erythroblastotic infants contain anti Rh agglutinins, tests are made against a series of bloods of known Rh type, only those bloods being selected which are compatible as to blood group. The sera should first be inactivated at 56° C. for 10 to 20 minutes, since with undiluted fresh sera the reactions may be inhibited. When sera giving distinct reactions are found, they can be used for typing bloods of any group provided that the isoagglutinins

⁸ Wiener *Arch Path* 32: 229 (1941), Wiener and Peters *loc cit*.

⁷ Levine, Burnham, Katzin and Vogel *Amer Jour Obst and Gyn* 42: 925 (1941).

⁸ Landsteiner and Wiener *Proc Soc Exp Biol and Med* 51: 313 (1942).

α and β , if present, are removed or neutralized. The author⁸ uses for this purpose saliva from secretors, prepared simply as follows: Saliva is procured from group A and group B individuals known to be secretors (cf. Chapter XVII) and pooled in the proportion of two parts A saliva to 1 part B saliva. This mixture is placed in a boiling water bath for ten minutes, centrifuged, and the supernatant, slightly opalescent fluid is pipetted off. The "purified" saliva obtained in this manner keeps for an indefinite period when stored in the refrigerator under sterile conditions. The pooled saliva is added to human sera in the proportion of 2 parts of serum to 1 part of saliva in order to neutralize the isoagglutinins present in the serum. Highly active sera may be diluted with saline solution for purposes of economy. The isoagglutinins anti-A and anti-B in human anti-Rh serum can also be removed, though less con-



Magnification 12

FIG. 55 TESTS FOR THE Rh FACTOR BY THE SEDIMENTATIVE TECHNIC

A and B Negative reactions, the inner light disc in B is due to a slight convexity in the bottom of the tube

C Faintly positive reaction

D Weak reaction

E and F. Typical positive reactions

[Reproduced from *Jour. Exp. Med.* 74: 309 (1941)]

veniently, by absorption with group A and group B blood. Still another procedure is to add solutions of the purified A and B substances such as the A substance from commercial peptone or pepsin (cf. page 338). However, the use of saliva is more convenient until both the group substances of animal origin become commercially available.

The Tests. When guinea-pig anti-Rh sera are used the clumping is not intense so that a special technic must be used. The tests are carried

⁸Wiener and Forer, *Proc. Soc. Exp. Biol. and Med.* 47: 215 (1941), Wiener, *Amer. Jour. of Clin. Path.* 12: 302 (1942)

out in small narrow tubes (inside diameter 7 mm) and the reactions are read by the so called sedimentive method of Landsteiner and Wiener. This technic is based on the observation that when a blood suspension is mixed with an indifferent serum, the erythrocytes settle out normally just like suspensions in saline solution so that a compact sediment with a sharply defined circular border is formed. On the other hand when the blood suspension is mixed with a serum containing an agglutinin acting on the erythrocytes, the sediment formed has a characteristic appearance (cf figure 55). In this way positive reactions can be recognized even in instances where on re-suspending the sediment hardly any agglutination is seen on microscopic examination.

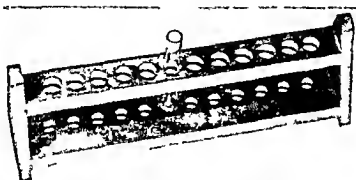


FIG 56 RACK USED FOR PERFORMING Rh TESTS BY
SEDIMENTIVE TECHNIC
(Reproduced by courtesy of the Certified Blood Donor
Service Jamaica N.Y.)

The blood suspensions for the tests should be prepared fresh washed with saline and re-suspended to form a 1 to 2 per cent suspension (in terms of blood sediment). Clotted whole blood or concentrated citrated blood from which the suspensions are prepared need not be as fresh provided they are obtained under sterile conditions and are stored in the refrigerator.

In carrying out the tests 2 drops of the diluted guinea pig serum are mixed with 1 drop of cell suspension and allowed to stand at room temperature. After 30 to 60 minutes when sedimentation is complete readings are taken by direct inspection of the bottoms of the tubes preferably with the aid of a hand lens. The readings are facilitated by the use of racks with holes beneath the tubes (cf figure 56). As already mentioned, negative bloods will show a circular deposit with a smooth edge, while positively reacting bloods have a wrinkled or granular deposit (cf figure 55). After the readings have been made the tubes may be allowed to stand and readings are taken again after re-

sedimentation Upon gentle shaking differences are usually noticeable on microscopic examination between positive and negative bloods, but, as already pointed out, at times the clumping is quite weak or even absent in spite of a distinctly positive sediment reading Naturally, positive and negative control bloods should be included in every set up Usually, a reliable diagnosis can be made from the very first reading at the end of 30 to 60 minutes based on the appearance of the sediment

While the guinea pig sera, as a rule react satisfactorily at room temperature, this is often not the case with human sera Human sera, particularly those obtained from mothers of erythroblastotic infants usually react most intensely at body temperature, as has been pointed out by Levine, in fact, with many such sera when tests are carried out at refrigerator temperature little or no clumping occurs Other human sera, however, are little affected by temperature and there are occasional sera, such as those found by Wiener and Peters, which react best in the cold In general, therefore, with unknown sera various temperatures should be tried

Diagnostic tests can be carried out in a manner similar to that described for the guinea pig sera at the temperature found optimal for the serum, usually 37°C, and the reactions are generally sufficiently developed within an hour If desired, the reactions can be accelerated by the centrifuge technic After 5 to 10 minutes incubation the tubes are centrifuged at low speed for 1 minute The sedimented cells are dislodged from the bottom of the tube by gentle shaking and the agglutination reactions read both macro and microscopically

HEREDITY OF THE RH FACTOR

Landsteiner and Wiener⁹ tested 60 families with 237 children for the presence of the Rh factor in the blood cells and obtained evidence that the property is transmitted as a simple Mendelian dominant The present author¹⁰ has more recently tested 40 additional families with 138 children The combined results of these two studies are summarized in table 50

From the families in which both parents are Rh negative, it is evident that the Rh agglutininogen is transmitted as a Mendelian dominant, 34 children from 7 such matings all proved to be Rh negative (Actually, in one family with 8 children tested by Landsteiner and Wiener, the oldest child was Rh positive On further investigation however, it was learned that he was a child of a former marriage) The high proportion of Rh- \times -Rh- matings in the table is due in part to selection

To account for the hereditary transmission of the Rh agglutininogen, Landsteiner and Wiener have postulated the existence of a pair of

⁹ *Jour Exp Med* 74 309 (1941,

¹⁰ Wiener and Sonn, *Genetics*, in press

allelic genes, *Rh* and *rh*, where the dominant gene *Rh* determines the presence of the factor, in analogy to other agglutinogens of human blood. Under this theory, Rh negative individuals are all homozygous, genotype *rh rh*, while Rh positive individuals could be either homozygous or heterozygous, genotypes *Rh Rh* and *Rh rh*.

The theory can be tested by calculating the distribution of the Rh factor among the children of the three sorts of matings and then determining how closely the observed values approach the expected ones.

TABLE 50
HEREDITY OF THE Rh AGGLUTINOGEN

Mating	Number of Families	Number of Children		
		Rh +	Rh -	Totals
Rh + × Rh +	73	248	16	264
Rh + × Rh -	20	54	23	77
Rh - × Rh -	7	0	34	34
Totals	100	302	73	375

This table includes 60 families with 237 children by Landsteiner and Wiener [*Jour Exp Med* 74: 309 (1941)] and 40 families with 138 children by Wiener and Sonn [*Genetics* March (1943)].

To make the computations it is first necessary to determine the relative frequencies of genes *Rh* and *rh* and of genotypes *Rh Rh* and *Rh rh*.

Since in the population examined the frequency of Rh negative individuals is 14 to 15 per cent

$$rh = \sqrt{0.145} = 0.381 \text{ or } 38.1 \text{ per cent} \\ \text{and } Rh = 61.9 \text{ per cent}$$

The proportion of heterozygous individuals among those positive for Rh is

$$\frac{2(Rh)(rh)}{(Rh)^2 + 2(Rh)(rh)} = \frac{2rh}{Rh + 2rh} = \frac{76.2}{138.1} = 55.2 \text{ per cent}$$

In the mating Rh + × Rh + Rh negative children can only occur when both parents are heterozygous genotype *Rh rh* and in such families one-fourth of the children should be negative. Accordingly the expected incidence of Rh negative children in the Rh + × Rh + matings is $\frac{1}{4} (0.552)^2 = 7.6$ per cent. The observed incidence 16 among 264 children or 6.1 per cent does not differ significantly from the calculated value.

In the mating Rh + × Rh - Rh negative children occur only in those matings where the Rh positive parent is heterozygous; half the children in such families being negative. The expected incidence of Rh negative children in Rh + × Rh - matings is therefore $\frac{1}{2} (0.552)$ or 27.6 per cent. The actual frequency 23 among 77 children or 29.9 per cent is only slightly higher than the calculated value.

Rh Factor and Sex The Rh factor is neither sex linked nor sex influenced in its hereditary transmission. This follows from the observa-

TABLE 51
FAMILY DATA DEMONSTRATING INDEFINITE ASSORTMENT OF GENES FOR Rh, BLOOD GROUPS AND M N TYPES

Family Number*	Mating (Genotypes)	Children**		
		U	V	Indeterminate
1 L & W #6	OOrrrh × 1 ₁ ORrrh	A ₁ Rh-	ORh-	A ₁ Rh+, ORh+
2 L & W #54	OOrrrh × 1 ₁ 1 ₂ Rrrh	A ₂ Rh+, A ₁ Rh-, A ₂ Rh+, A ₁ Rh+	A ₁ Rh+	
3 W #1	BOrrrh × OOrrrh	ORh-, ORh-		BRh+, ORh+, BRh+, ORh+, BRh+
4 W #7	OOrrrh × 1 ₁ O Rrrh	A ₁ Rh+, ORh-, A ₁ Rh+	A ₁ Rh-	
5 W #18	OOrrrh × 1 ₁ BRrrh	A ₁ Rh-, BRh+	BRh-	
6 W #19	OOrrrh × 1 ₁ OORrrh	A ₁ Rh-, A ₁ Rh-, A ₁ Rh-	A ₁ Rh+, ORh-	
7 W #39	1 ₂ ORrrh × OORrrh	ORh-, ORh-	A ₁ Rh-	ORh+
1 L & W #4	MNRrrh × NNRrrh	MNRh-, NRh+, NRh+	NRh-, NRh-	MNRh+, MRh+
2 L & W #6	MNRrrh × MMRrrh	MNRh-	MRh-	MNRh-
3 L & W #13	MNRrrh × MNRrrh	MRh+	MRh-	MRh+, MNRh+, MNRh+, MNRh+
4 W #1	MNRrrh × MMRrrh	MRh-, MRh-		MNRh+, MNRh+
5 W #19	MVrrrh × MNRrrh	MRh-, MRh-	NRh-, NRh-	MRh+, MRh+
6 W #26	MNRrrh × MNRrrh	MNRh-	MRh-	

* I & W = I and Wiener and Wiener, *loc cit*, W = Wiener, unpublished

** For the significance of classes U, V and indeterminate, cf page 243

tions of Landsteiner and Wiener that the distribution of that factor is the same in the two sexes, not only in the general population, but also among the children in the families with Rh-positive fathers and Rh-negative mothers, while, if sex-linkage were present, offspring only (or predominantly) of one sex should exhibit the character.

Relationship between the Rh, A-B and M-N Properties As has already been mentioned, Landsteiner and Wiener found that in the general population, the distribution of the Rh factor does not differ significantly in any of the four blood groups or in the three M-N types

For determining the linkage relations of the Rh property with the blood groups and M-N types, only those families can be used in which one of the parents is doubly heterozygous (cf page 243). For the blood groups this includes families with one parent of genotype $ABRhrh$, $AORhrh$ or $BORhrh$, and if tests for the subgroups are made also A_1A_2Rhrh . For studying the linkage relations of Rh and M-N, only families with one parent of genotype $MNRhrh$ can be used. The families among the 100 summarized in table 50 which give significant information with regard to linkage are listed in table 51.

With regard to the linkage relations of Rh and A-B, it will be seen that of a total of 25 children from 7 families 8 children fall into class V which does not differ significantly from the number expected under the assumption of independent assortment (6.9 ± 0.9).¹¹ Similarly in the six families yielding information as to the linkage relations of Rh and M-N 6 out of 17 children fall into class V the expected number being 3.9 ± 0.9 .

Based on these results it appears that the Rh factor is probably inherited independently of A-B and M-N (or at least it is not closely linked to either of these properties).

Development and Constancy of the Rh Type While systematic studies have not yet been carried out, it seems certain that the Rh type is fully developed at birth, since the reactions of the blood of newborn infants are indistinguishable from those of adults. That the Rh type is a constant characteristic of the individual, as would follow from its heredity, has been confirmed by tests made over a period of three years.

That the Rh factor is already present in the blood cells during fetal life would follow from the observations on isoimmunization against the Rh property during pregnancy (cf page 369). Direct evidence supporting this conclusion has been obtained by testing fetal blood.¹²

SUBDIVISIONS OF THE RH-POSITIVE TYPE

With the aid of standard anti Rh sera human beings are divided into only two types, Rh positive and Rh negative the latter comprising about 15 per cent of the white population in cities like New York. The situation is actually more complicated due to the existence of more than one variety of Rh agglutinin.

Shortly after the discovery of the Rh agglutinin Wiener¹³ obtained from a patient who had had hemolytic reactions to blood transfusions a serum which con

¹¹ For method of computing this value cf Wiener, *Genetics* 17: 335 (1932).

¹² Bornstein and Israel, *Proc. Soc. Exp. Biol. and Med.* 49: 718 (1942). However, cf Fisk and Foord, *Amer. Jour. Clin. Path.* 12: 545 (1942).

¹³ *Arch. Path.* 32: 229 (1941).

tained an irregular agglutinin acting on about 70 per cent of bloods. That the property defined by this serum is related to the Rh agglutino-gen was established by the fact that bloods reacting negatively with standard anti Rh sera almost regularly gave negative reactions with this special serum¹³ which later on proved to be of less frequent occurrence. This situation seemed at first to resemble the subdivisions of group A especially when Levine¹⁴ encountered a serum from an Rh positive post partum patient which agglutinated all Rh negative bloods and in addition those Rh positive bloods which were not agglutinated by the special variety of anti Rh

TABLE 52

COMPARISON OF REACTIONS OF A STANDARD AND A SPECIAL ANTI RH SERUM
[Landsteiner and Wiener, *Jour Exp Med* 74 309 (1941)]

Reactions with standard human anti Rh serum	Reactions with special anti Rh	
	Positive	Negative
Positive	31	8
Negative	1	11

serum in analogy to the reactions of anti O serum with bloods of group O and subgroup A₁.

Upon further study it became clear however that the subdivisions of the Rh positive type are not entirely comparable to the subdivisions of group A because the former are more complicated. Thus occasional bloods were found which failed to react with the standard anti Rh serum but reacted nevertheless with the anti Rh serum giving fewer positive reactions¹⁵. In a series of 52 blood samples Landsteiner and Wiener found 31 that were agglutinated by both antisera 11 that were negative with both sera 8 that were agglutinated by the standard anti Rh serum but not by the other anti Rh serum while 1 blood was agglutinated by the special serum even though it was negative with the standard serum (cf table 52). This established that the agglutinins in the two sera are qualitatively different though related and that they define 4 types of human blood. It is proposed to designate the agglutinin in the standard anti Rh serum (giving about 85 per cent positive reactions) as anti Rh₁ while the agglutinin in the less common serum (giving about 70 per cent positive reactions) is designated as anti Rh₂ (cf table 52a) to conform with the order in which they were found.

Levine et al¹⁶ have recently observed a third type of human anti Rh serum which gives about 87 per cent positive reactions with bloods from white individuals. Levine^{16a} has studied the relationships between the three varieties of anti Rh sera and his findings are included in table 52a. It is evident that serum of type 3 behaves like a mixture of sera of types 1 and 2 and in absorption experiments Levine did obtain two distinct agglutinin fractions giving reactions corresponding to anti Rh₁

¹³ Wiener, *loc cit*, Landsteiner and Wiener *Jour Exp Med* 74 309 (1941)

¹⁴ Levine meeting of N Y Path Soc Jan 22 1942. However, Levine has suggested that the property detected by this serum designated by him as Hr may be allelic to Rh analogous to the properties M and N.

¹⁵ Landsteiner and Wiener *loc cit*, Levine personal communication.

¹⁶ Levine Burnham Katzin and Vogel *Amer Jour Obst and Gyn* 42 925 (1941)

^{16a} Personal communication Cf Davidsohn and Toharsky, *Amer Jour Clin Path* 12 434 (1942)

and anti Rh. For this reason it is proposed to designate the agglutinins in the third sort of anti Rh serum as anti Rh₁, (cf table 52a) Incidentally sera of types 1 and 3 are distinguished either by direct tests on bloods of incidence 2 per cent in table 52a or by absorption tests with bloods of incidence 15 per cent

The reactions could be explained most simply by assuming the existence of two qualitatively different agglutinogens Rh₁ and Rh₂ in the blood cells corresponding to the agglutinins anti Rh₁ and anti Rh₂. The four sorts of blood would then have

TABLE 52a

RELATIONSHIP BETWEEN THE REACTIONS OF DIFFERENT TYPES OF ANTI Rh SERA

Anti Rh Sera	Originally Described by	Designation of Agglutinins in Serum*	Approximate Per Cent Positive Reactions	Reactions of Various Bloods Among White Individuals			
				About 70%	About 15%	About 2%	About 13%
Type 1 (standard anti Rh)	Landsteiner and Wiener	anti Rh ₁	85	Pos	Pos	Neg	Neg
Type 2		anti Rh ₂	72	Pos	Neg	Pos	Neg
Type 3		anti Rh ₁	87	Pos	Pos	Pos	Neg
So-called anti Hr serum	Levine, Javert and Katzin	anti Hfr	—	Neg or Weakly Pos	Pos	Neg	Pos

* This designation was adopted after discussion with Drs Landsteiner and Levine

(the composition Rh₁Rh₂, Rh₁, Rh₂, Rh negative respectively) This assumption would imply the existence however of two corresponding genes Rh₁ and Rh₂, which would have to be either independent linked or allelic The first two possibilities are excluded since the product of the frequencies Rh₁Rh₂ × Rh negative is much greater than Rh₁ × Rh₂. Moreover the existence of allelic genes Rh₁ and Rh₂ in individuals whose bloods react with both anti Rh₁ and anti Rh₂ would necessitate that this class not exceed 50 per cent while the actual frequency is 70 per cent Accordingly the observations are best explained by assuming the existence of 3 qualitatively different Rh agglutinogens instead of only 2 one type reacting with anti Rh₁ serum but not anti Rh₂, a second reacting with anti Rh₂ but not anti Rh₁, and a third reacting with both sorts of anti Rh sera (vide Landsteiner¹⁷)

While the sera of types 1, 2 and 3 (table 52) are those most often encountered Levine has found a few human sera from mothers of erythroblastic infants which give divergent reactions with rare bloods indicating the possible existence of still further varieties of the Rh agglutinin Because of the rarity both of the sera and of the bloods these have not yet been thoroughly studied

Some preliminary studies by Wiener and Landsteiner indicate that the varieties of Rh agglutinin are hereditary

¹⁷ Landsteiner K. Specificity of Serological Reactions pp 140-144 Charles C Thomas, Springfield, Ill. (1936)

CHAPTER XVI

FURTHER INDIVIDUAL DIFFERENCES IN HUMAN BLOOD

BY MEANS OF the five agglutinogens A_1 , A_2 , B, M and N, and the two common varieties of anti-Rh agglutinins, 72 distinct types of human blood can readily be identified.¹ There is evidence for the existence of still other specific agglutinogens in human red blood cells. Since each additional independent agglutinin doubles the number of subdivisions

TABLE 53
EXPERIMENT SHOWING THE INDIVIDUALITY OF THE BLOOD
(After Landsteiner and Levine)

	Blood								
	v d Sch	Ldst	Henry	Mock	Ph L	Hdy	Bl	Kr	A W
Group	O	O	A	O	A	A	A	O	O
Reaction for M	+ ±	-	++ ±	++ ±	-	++ ±	++ ±	-	+ ±
Reaction for N	+	++	-	+	++ ±	+	-	++ ±	+ ±
Serum 1219 A_2 (agglutinin α_1)	-	-	+	-	-	+	-	-	-
Serum A, no 740†	+	tr	-	±	+	+	tr	+ ±	-
Serum B, no 2038†	±	-	*	-	*	*	*	+	-
Serum B, Menz†	-	+	*	-	*	*	*	-	±

* Isoagglutination † Sera containing irregular isoagglutinins

of human blood, an individuality of the blood reminiscent of the individuality of the finger-prints is conceivable. Such a view has been suggested by Landsteiner.² In this connection, an experiment performed by Landsteiner and Levine³ may be cited. The bloods of nine individuals working in their laboratory were tested by means of immune agglutinins and certain atypical human agglutinins. When a second set of specimens labelled by numbers only was tested with the same reagents, it was not difficult to determine to which individual each of the specimens belonged (cf. table 53).

¹ And this does not take into account rare bloods of subgroup A_3 and A_3B , or of type MN_2 , etc

² *Science* 73: 403 (1931)

³ *Jour Immunol* 17: 1 (1929)

However, factors in addition to those discussed in the preceding chapters are not yet available for general use because of the difficulty of preparing or securing the proper reagents. Indeed, with only one or two exceptions (agglutinogens P and Q) the results have not been perpetuated by the original observers by establishing standard bloods so that it is not possible now to compare the reactions of any new sera that may be obtained with those previously reported in the literature.

In this chapter will be presented, first what is known regarding the agglutinin P, then some remarks concerning individual differences of human blood in general.

AGGLUTININ P OF LANDSTEINER AND LEVINE

In 1927, when Landsteiner and Levine¹ prepared the first immune anti M and anti N rabbit sera, they also obtained sera which defined still another property in human blood, designated by them as agglutinin P. Isoantibodies against P occur occasionally in normal human sera² and rarely as a result of isomunization in patients receiving repeated blood transfusions.³ While the possibility exists that this factor could on rare occasions give rise to hemolytic transfusion reactions, to date no such reactions have been reported (cf. page 118). If the technic can be worked out more satisfactorily the agglutinin P may find application in forensic medicine as an additional means of individualizing human blood and because of its hereditary nature also for the exclusion of parentage (cf. page 391).

Technic of Testing for P Agglutinin. The main difficulty is that no reliable method has yet been devised whereby reagents of satisfactory potency and uniform specificity can be obtained regularly. The immune rabbit serum obtained by Landsteiner and Levine gave strong reactions with many bloods, but there were transitions between positively and negatively reacting bloods, moreover, attempts to produce the anti-sera again were either negative or yielded reagents less satisfactory than the original serum.⁴

In occasional human sera as already mentioned Landsteiner and Levine encountered irregular isoagglutinins giving reactions corresponding to the P factor. Aside from the rarity of such sera these had the disadvantage that the reactions are considerably weaker than those

¹ *Jour. Exp. Med.* 47: 57 (1928).

² Landsteiner and Levine *Jour. Immunol.* 17: 1 (1929). Nigg *Jour. Immunol.* 19: 1 (1930), Wiener unpublished observations.

³ Wiener and Peters *Ann. Int. Med.* 13: 2306 (1940). Wiener *Amer. Jour. Clin. Path.* 12: 307 (1942).

⁴ Landsteiner, Strutton and Chase *Jour. Immunol.* 27: 469 (1934). Schiff *Deutsche Zeitschr. f. d. ges. gerichtl. Med.* 21: 404 (1933).

of the immune sera. In fact, the agglutinin in these sera was designated at first as extra-agglutinin 1 by Landsteiner and Levine, because so many more negative reactions were obtained that the relationship to the anti-P immune serum was not immediately recognized. Nigg⁸ has also described two human sera which were later found to contain natural anti-P isoagglutinins, and 3 or 4 such sera have been encountered by the present author during the past 10 years. Two post-transfusion sera referred to the author for study were found to contain anti-P isoagglutinins, apparently resulting from isoimmunization (cf. page 65). Although the agglutinins in these sera were of low titer,

TABLE 54

DEMONSTRATION OF THE AGGLUTINOGEN P WITH THE AID OF NORMAL ANIMAL SERA
(After Landsteiner and Levine)

Serum	Num ber	Cells Group O												
		1	13	31	32	33	34	35	36	37	38	43	44	74
Rabbit	71	0	+ ±	tr	0	0	±	+	0	+	0	tr	tr	0
Horse	5	0	+ ±	tr	0	0	±	+	0	+	0	0	±	0
Horse	10	0	+ ±	0	0	0	+	+ ±	0	+	0	0	±	tr
Pig	6	f tr	+ ±	±	f tr	0	±	+	tr	+	0	tr	+	tr
Human group B (unab- sorbed)	64	0	+	0	0	0	f tr	+	0	+	0	0	±	0

The 4 animal sera were absorbed with blood no. 38, group O. For comparison the tests with a human serum (group B) containing extra agglutinin 1 are given.

a sharp differentiation between P+ and P- blood could be made in tests at refrigerator temperature.

Suitable reagents for testing for property P are obtained most readily from normal animal sera⁹ (cf. table 54) since such sera, particularly from pigs and horses, not infrequently contain anti-P agglutinins. Dahr¹⁰ encountered a normal pig serum containing anti-P agglutinins of exceptionally high titer (256), permitting a sharp distinction between P+ and P- blood and enabling him to carry out extensive investigations on the heredity of the P agglutino-gen (cf. page 259).

Animal sera must first be treated with human cells to remove any hetero-agglutinins that may be present, also group agglutinins such as anti A, anti B or

⁸ *Jour Immunol* 19: 1 (1930)

⁹ Landsteiner and Levine, *Jour Immunol* 20: 179 (1931)

¹⁰ *Klin Woch* 18: 806 (1939)

anti O. The technic recommended by Landsteiner and Levine is to inactivate the serum dilute it with an equal volume of saline solution and mix it with half its volume of pooled washed packed P negative cells of groups O, A₁ and B. After the mixture has stood in the refrigerator overnight the serum is separated and tested against standard P+ and P— blood. If any reaction still occurs with the latter the absorption is repeated usually with a smaller quantity of cells. If bloods of known P types are not available by systematically absorbing a number of animal sera with different human bloods reagents can be obtained which give distinct and parallel reactions with selected human bloods independent of the blood groups and these as a rule will correspond to agglutininogen P.

Once suitable reagents have been obtained, the tests offer little difficulty, these being carried out like ordinary blood grouping tests.

TABLE 55
HEREDITY OF THE P AGGLUTINOGEN
(After Dahr and Zehner)

Mating	Number of Families	Number of Children		
		P+	P—	Totals
P+ × P+	249	677	79	756
P+ × P—	134	286	179	465
P— × P—	34	(4)	94	98
Totals	417	967	352	1319

This table includes the following studies

Dahr 112 families with 434 children

Dahr, Offe and Weber 98 families with 306 children

Brinkmann 47 families with 129 children

Nussbaum 40 families with 144 children

and Wahlen 120 families with 306 children

Heredity of the P Agglutininogen The first studies on the heredity of the P agglutininogen were handicapped by the lack of potent reagents. Using human natural anti P agglutinins in a study on 103 families with 498 children, Landsteiner and Levine¹¹ demonstrated the hereditary nature of the P type, but because of the frequent occurrence of indefinite reactions were unable to determine the exact mechanism. With the aid of more satisfactory reagents prepared from normal animal sera Landsteiner and Levine¹² tested 71 families and obtained evidence which would seem to indicate that the P factor is transmitted as a Mendelian dominant, inasmuch as they encountered 4 families with P negative parents in which all 18 children proved to be P negative.

The comprehensive investigations of Dahr and his collaborators¹³

¹¹ *Jour Immunol* 18 87 (1930)

¹² *Jour Immunol* 20 179 (1931)

¹³ Dahr *Ztschr f Immunitat* 97 170 (1939), Dahr Offe and Weber *Ztschr*

using the potent anti P pig serum referred to above, are summarized in table 55. It will be seen that among 1319 children there are only 4 exceptions to the theory that the P agglutinin is inherited as a simple Mendelian dominant by a pair of genes, P and p . In not one of the four cases could the possibility of illegitimacy be excluded, moreover, in a study of 150 families with 751 children, Hausen¹⁴ did not encounter a single exception.

The theory can be tested further by determining how closely the distribution of the P agglutinin among the children of the different matings agrees with the predicted values. This is done by the same method used for agglutinin Rh (cf. page 250).

Among 3530 individuals examined Dahr and his collaborators found that 2684 or 76.03 per cent were P positive and 846 or 23.97 per cent P negative.

Therefore $p = \sqrt{0.2397} = 0.4896$

and $P = 1 - p = 0.5104$

Accordingly genotype $Pp = 0.4998$

and genotype $PP = 0.2605$

In the mating $P+ \times P+$ the expected frequency of P negative children is $\frac{1}{4} \left(\frac{0.4998}{0.7603} \right)^2 = 0.103$ or 10.3 per cent. The observed frequency 79 among 756 children or 10.4 per cent agrees closely with the predicted value.

In the mating $P+ \times P-$ the calculated frequency of P negative children is $\frac{1}{2} \frac{0.4998}{0.7603} = 0.3287$ or 32.9 per cent. The observed value 179 among 465 children or 38.5 \pm 1.5 per cent is higher but the possibility that the difference (about 3 times its probable error) is due to chance cannot be excluded.

Variations in the P Agglutinin. In tests on human bloods with anti P reagents prepared from various animal sera, Landsteiner and Levine¹⁵ observed that while on the whole the reactions ran parallel, there were some discrepancies when different sera were used. These authors concluded that the so called property P is not a single entity but comprises a group of related agglutinogens. In this respect agglutinin P would not differ from certain other individual agglutinable properties of human blood, in particular A and Rh (cf. Chapter XII and page 252).

With regard to quantitative differences the present author¹⁶ has observed in tests made with natural and immune human anti P sera that human bloods can be divided into three classes according to the intensity of the reactions (cf. table 56), namely, strong, moderate and completely negative reactors. Whether the differences in intensity of agglutination are due to homo- and heterozygosity (cf. page 227) or to

¹⁴ *f. Rassenphysiol.* 11: 78 (1940). Dahr and Zehner. *Deutsch. med. Woch.* 67: 71 (1941).

¹⁵ Cited after Dahr and Zehner *loc. cit.*

¹⁶ *Jour. Immunol.* 20: 179 (1931).

¹⁷ *Wiener Blood Groups and Blood Transfusion* p. 182. 2nd edition (1939).

qualitative differences in the P agglutinin can only be determined by family studies

The investigations of Furuhashi and Imamura¹⁷ on the so-called ag

TABLE 56

CLASSIFICATION OF INDIVIDUALS INTO THREE GROUPS BY THE REACTIONS OF THE BLOOD WITH ANTI P SERUM

Blood of	Reactions with Absorbed Pig Serum		Results
	1 hr room temp	8 hrs ice box	
A Ho	-	-	P neg
B Zu	+ ±	++ ±	P strong
B La	-	++	P medium
B Wol	-	+	P medium
M Le	-	-	P neg
Miss F	-	-	P neg
G Go	-	-	P neg
B Ka	-	-	P neg
G Pe	-	-	P neg
H As	+ ±	+++	P strong
R Se	-	-	P neg
A Sc	+	++	P medium
L Wi	±	+ ±	P medium
S Ma	+	++	P medium
F Di	-	+ ±	P medium
J F	-	++	P medium
J Ma	+	+ ±	P medium
H Do	-	++	P medium
A Bu	-	-	P neg
L Fr	-	+	P medium
B Fi	++	++ ±	P strong
A Wi	±	+ ±	P medium
C Ed	+ ±	++ ±	P strong

This table also illustrates the importance of performing these tests under optimal conditions, since when the tests are not sufficiently sensitive false negative reactions are obtained

glutinin Q are of interest. This property was determined with the aid of an absorbed normal pig serum. The agglutinin Q has proved to be unrelated to the agglutinogens A, B, M and N but there is a marked correlation between the reactions for P and Q, as is shown in table 57. For example, if Dahr's results are compared with those expected on the assumption that P and Q are independent, then we obtain for χ^2 the very high value of 39.5. The situation here is very similar to that

¹⁷ *Jap Jour Genetics (Japanese)* 12: 50 (1936)

which exists with regard to the Rh factor, where the reactions of two different varieties of anti Rh sera also make possible the separation of human beings into 4 classes (cf page 253) Accordingly, the so-called agglutinin Q is most likely one variety of the agglutinogens designated as P Investigations on the agglutinin Q in large series of families revealed that the property is inherited as a simple Mendelian dominant^{18 19 20}

P Agglutinin in Twins Dahr Offe and Weber²¹ have tested 134 pairs of monozygotic and 188 pairs of dizygotic twins for the P factor While the bloods of identical twins invariably gave corresponding reactions 43 of the 188 pairs of

TABLE 57
RELATIONSHIP BETWEEN AGGLUTINOGENS P AND Q

Investigator	Number of Bloods Giving Reactions				Totals
	P+Q+	P+Q-	P-Q+	P-Q-	
Imamura*	16	7	4	11	38
Wiener†	7	0	0	3	10
Dahr‡	57	5	4	17	83

* *Hanraigaku Zasshi* (Japanese) 9 580 (1935)

† Unpublished data (1939)

‡ *Ztschr f Rassenphysiol* 11 78 (1940)

fraternal twins gave discordant results Such findings were to be expected in view of the hereditary nature of the P agglutinin and they demonstrate that almost one fourth of fraternal twin pairs can be recognized by testing for the P factor

Relation of the P Agglutinin to A B, M N, Rh and Sex As has already been mentioned, Landsteiner and Levine found that the P agglutinin is distributed independently of the blood groups M N types, and sex In addition, Landsteiner and Wiener²² have recently demonstrated that the properties Rh and P are distributed independently of each other in the general population

In the course of a study on the heredity of the blood groups and M N types the present author forwarded the blood samples of a number of families to Dr Karl Landsteiner and Dr Philip Levine (1931) who tested them for agglutinin P Analysis of these data²³ suggested that the genes for factor P are on a different chromosome or at least not closely linked to A B O or M N Further

¹⁸ Furubata and Imamura *Jap Jour Genetics* (Japanese) 11 91 (1935)

¹⁹ Imamura *Hanraigaku Zasshi* (Japanese) 9 589 (1935)

²⁰ Imamura and Suzuki *Jap Jour Genetics* (Japanese) 12 50 (1936)

²¹ *Ztschr f Rassenphysiol* 11 78 (1940)

²² *Jour Exp Med* 74 309 (1941)

²³ Wiener cited by Zieve Wiener and Fries [*Ann Eug* 7 163 (1938)] Analysis of the data of Dahr Offe and Weber yielded similar results

more the property P is not sex linked. No data are available as yet with regard to the linkage relations of properties Rh and P. If these prove to be independent then there will be four chromosomes marked with genes for blood factors available for linkage studies in human heredity (cf page 373)

INDIVIDUAL DIFFERENCES IN HUMAN BLOOD GENERAL CONSIDERATIONS

For the demonstration of individual differences in human blood four sorts of reagents are available namely natural and immune animal sera and natural and immune human sera

Individual Blood Differences Demonstrable with Animal Sera As has been explained in the description of the tests for M and N when specific reagents against a particular agglutinin are to be prepared from animal sera a preliminary absorption is ordinarily necessary to remove any species specific agglutinins which may be present

When endeavoring to find new agglutinogens in human erythrocytes by means of natural or immune animal sera a number of difficulties must be overcome (1) Antibodies against agglutinogens present in a high percentage of human bloods may not be detected for the reason that all the absorbing bloods may contain the agglutinin in question and will remove the corresponding specific agglutinin from the serum (2) On the other hand in the case of rare agglutinogens no blood possessing the property may be encountered (3) The preliminary absorption necessary to remove the species agglutinins may non specifically remove or weaken the specific agglutinins as is the case for the agglutinin N (cf page 224) A fourth difficulty arises from the fact that the sera of animals of different species or even of the same species vary in their natural agglutinins and that different animals do not respond in the same way to immunization Consequently it may not be easy to reproduce the results of experiments which seem to indicate the existence of new properties Probably the greatest obstacle is that agglutinable substances responsible for individual differences may fail to evoke the production of immune antibodies For example in human beings, the transfusion of type M blood into type N individuals or vice versa only extremely rarely stimulates the formation of isoantibodies

The first systematic search for individual differences in human blood independent of the blood groups was made by von Dungern and Hirschfeld¹ in 1910 These authors examined a large series of animal sera from various species in an attempt to find natural individual agglutinins for human bloods Each serum was absorbed with different human bloods and tested against a number of individual blood samples By means of the absorbed sera it could again be established that many of these animal sera contained natural agglutinins specific for A and B² Furthermore in

¹ *Ztschr f Immunstat* 8 526 (1911)

² Cf Landsteiner *Munch med Woch* 49 1905 (1902)

some animal sera, agglutinins apparently specific for bloods of group O could be demonstrated (cf page 201) Von Dungern and Hirschfeld believed that their experiments also revealed a number of differences that would make possible an individual diagnosis of every human blood. However the reactions did not determine sharply defined properties nor were they always reproducible. It is not unlikely that the differences were in part caused by accidental quantitative variations so frequent in serological work.²⁰ Subsequent experiments (see below) tended to corroborate the occurrence of various (mostly minor) blood differences demonstrable by absorbed normal (and immune) animal sera, but there is no technic available whereby these reactions can be put to practical use. It is very likely that some of the stronger re-

TABLE 58

INDIVIDUAL DIFFERENCES IN HUMAN BLOOD DEMONSTRATED BY
MEANS OF NORMAL CAT SERA
(After Landsteiner and Witt)

Serum Absorbed with Corpuscles		Tested against Corpuscles									
		Group O					Group A		Group B		
		151	155	156	157	159	152	158	153	154	160
Group O	151	0	0	0	0	0	++	+++	+	+	+
	155	+	0	0	0	±	+++±	+++±	+	+	+
	156	+	0	0	0	0	+++	+++±	+	+	+
	157	+	0	0	0	0	+++±	+++	+	+	+
	159	+		0	0	0	+++	+++±	+	+	+
Group A	152	+	±	±	tr	tr	0	0	+	+	+
	158	+	tr	0	0	0	0	0	+	+	+
Group B	153	+	+	0	tr	tr	++	+++±	0	0	0
	154	+	0	0	0	0	++++	+++±	±	0	0
	160	+	±	tr	±	+	+++±	++	+	+	0

actions observed by von Dungern and Hirschfeld correspond to the type P of Landsteiner and Levine.

An experiment performed by Landsteiner and Witt²¹ along similar lines may be cited. The pooled sera of 10 cats were absorbed with washed sediment of 10 different human bloods and tested against cell suspensions of each of the absorbing bloods. From table 58 it is obvious that the pooled sera contain specific agglutinins against the agglutinogens A and B so that the groups of the 10 bloods could be determined from the reactions. The 3 bloods of group B showed some differences, the 2 group A bloods were alike and of the 5 group O bloods 4 showed only slight variations (accidental?), but one differed markedly. This blood apparently contained an agglutinin independent of the agglutinogens A and B. Though this

²⁰ This is substantiated by the experiment given in the table on page 546 of the paper by von Dungern and Hirschfeld.

²¹ *Jour Immunol* 11: 221 (1926).

particular experiment was clean-cut several attempts to reproduce it have been unsuccessful thus emphasizing the difficulties mentioned above

The blood properties described in the following paragraphs are discussed for the sake of completeness, but are not easily accessible to investigation, and for the most part have only been studied by the original authors

Andresen²⁸ has described an agglutinable property of human blood detected with an immune serum which he obtained from a rabbit that had been injected with OM blood When an 1:20 dilution of this serum was suitably absorbed it was found to contain in addition to potent anti M agglutinins, another agglutinin designated by Andresen as anti λ 94 per cent of a series of 200 human bloods were found to contain the property λ The difficulty of finding blood lacking the property λ which can be used for absorbing immune sera may account for the fact that antibodies for λ were not previously detected and will prove an obstacle to further studies

Landsteiner, Strutton and Chase²⁹ injected rabbits with negro blood which gave a strong reaction for P and obtained an immune serum which after absorption gave reactions defining a new agglutinin This agglutinin in contrast to property λ is rare It is much more common in negroes than in whites since it was found in only 2 out of 387 white individuals and in 14 out of 191 negroes³⁰ Remarkably all the bloods that gave distinctly positive reactions belonged to types N or MN

It is possible that additional differences in human blood may be discovered with the aid of normal or immune sera from animals other than those already used Thus Schiff³¹ has described a new agglutinin (H) detectable with the aid of an immune sheep serum which is inherited as a simple mendelian dominant Using normal eel serum Sugishita³² found that some of the sera agglutinate all human blood with approximately equal intensity other eel sera act most strongly on group O cells With the aid of sera of the latter sort bloods from groups A B and AB could be divided into two types Those bloods which are agglutinated by higher dilutions of eel serum were designated as type E those agglutinated only by undiluted or at least not highly diluted serum as type e From family studies it seems that the properties E and e are hereditary qualities³³ closely related to if not identical with those demonstrable with anti O sera (cf page 201)

²⁸ *Ztschr f Immunitat* 85 227 (1935)

²⁹ *Jour Immunol* 27 469 (1934)

³⁰ In one experiment performed with serum kindly supplied by Dr Landsteiner the writer tested a series of over 25 bloods from white individuals and included as a control blood from the negro used for the immunization A positive (strong) agglutination reaction occurred with the control blood but none of the other 25 bloods showed any agglutination.

³¹ *Die Naturwiss* 20 658 (1932)

³² Sugishita *Ju enka Zasshi* (Japanese) 40 1938 (1935)

³³ Sugishita *ibid* 40 3367 (1935)

Ottenssooser³⁴ has described attempts to demonstrate individual differences in human blood with the aid of polyvalent immune sera which in his opinion demonstrate an almost complete individuality. Rabbits were given injections of the pooled blood from almost 500 different persons. The immune rabbit sera were pooled and incompletely absorbed with a variety of human bloods. The tests were made on slides and the time noted when agglutination appeared with various samples of blood. A similar technic was used by Ottenssooser for testing human blood with normal animal sera. Here similar remarks apply as have been made with regard to the experiments of von Dungern and Hirsfeld. Similar experiments have been carried out by Lattes and Domenici³⁵ but further investigations are needed to establish the real value of this method.

Individual Differences in Human Blood Demonstrable with Human Sera. The demonstration of individual differences in human blood by means of normal or immune isoagglutinins is simpler than with animal sera, insofar as no preliminary absorption is necessary to remove species agglutinins. As has been pointed out, a small percentage of normal human sera contains irregular isoagglutinins that produce reactions of minor degree (cf. page 44).

Such agglutinins have been reported from time to time since the blood groups were first described, but in most cases, they were not systematically studied. A rather active irregular agglutinin (titer 1:8) found by Ottenberg and Johnson³⁶ in the serum of an individual belonging to group B, was somewhat more thoroughly investigated. This isoagglutinin, unlike most other irregular agglutinins, was active even at 37°C. The agglutino-gen defined by this serum was also studied by Landsteiner and Levine,³⁷ who found that it bore no relation to any of the previously known agglutinogens. Some of the reactions produced by the serum are shown in table 53 (serum B, Menz).

Guthrie and his coworkers³⁸ described a serum of group O that acted on only one of 18 bloods of the same blood group. Jones and Glynn³⁹ made a systematic study of the sera of 40 normal individuals by testing each serum against the blood cells of the other 39 individuals. They found that six of the sera gave atypical reactions. Among 3500 sera, Thomsen⁴⁰ found 32 that gave abnormal reactions.

Landsteiner and Levine⁴¹ investigated this problem thoroughly and

³⁴ *Ztschr f Immunitat* 89: 260 (1936).

³⁵ *Hematologica* 18: 543 (1937). Also see Hausbrandt *Hematol* 18: 593 (1937).

³⁶ *Jour Immunol* 12: 35 (1926).

³⁷ *Jour Immunol* 17: 1 (1929).

³⁸ Guthrie and Huck. *Bull Johns Hopkins Hosp* 34: 57-80, 128 (1923). *ibid* 35: 23 (1924). Guthrie and Pessl. *ibid* 35: 33, 81, 126 (1924).

³⁹ *Jour Pathol and Bacterial* 29: 203 (1926).

⁴⁰ *Ztschr f Immunitat* 57: 301 (1928).

⁴¹ *Jour Immunol* 20: 179 (1931).

systematically. The most frequent of the irregular isoagglutinins found by these authors were related to the subgroups of group A and group AB (cf. page 45). Other sera were found which gave reactions unrelated to the blood groups. Of these, the most common were those containing the so-called extra-agglutinin 1 (cf. table 59), whose reactions corresponded roughly to those of immune anti-P sera. The reactions of the other sera containing atypical isoagglutinins were not classified.

A number of the sera containing irregular agglutinins were re-examined by Landsteiner and Levine several times over a period of a year. The results on the whole were consistent. In some cases, however, there were variations in the intensity of the reactions so that weak agglutinations were not reproducible on re-

TABLE 59
REACTIONS WITH HUMAN SERA CONTAINING EXTRA AGGLUTININ 1
(After Landsteiner and Levine)

Serum Number	Group	Corpuscles of Group O											
		33	34	46	250	299	538	901	915	916	938	974	1021
299	O	0	+	f tr	+	0	0	0	f tr	0	0	+	+
1172	A	0	+	f tr	+	tr	tr	tr.	f tr	0	0	+	±
1048	B	f tr	+	tr	+	0	0	0	0	0	0	+	±

examination. The stronger sera remained active when kept in the ice box for months whereas in weaker sera deterioration was noticeable after a shorter time.

Evidence for the hereditary character of the agglutino-gen discovered by Ottenberg and Johnson has been furnished in family studies by Landsteiner and Levine.⁴²

The present author⁴³ has recently encountered a human serum containing a natural isoagglutinin reacting with an extremely rare agglutino-gen in a human blood. When matching the bloods of a group B patient and donor, strong agglutination was observed in the mixture of donor's serum and patient's cells. The donor's serum, which contained potent anti-A agglutinins did not give a single abnormal reaction in tests on hundreds of other blood samples of all groups. Moreover, the patient's blood cells reacted perfectly normally when tested with numerous other human sera, being agglutinated uniformly by group A sera but not by group B sera, and the patient's serum contained a typical anti-A agglutinin. Accordingly, the abnormal agglutination reaction observed was apparently due to an unusual agglutino-gen in the patient's cells and the corresponding rare iso-agglutinin in the donor's serum. The agglutino-gen was not present in the blood cells of any of the patient's three group B children.

In general, the demonstration of individual differences aside from the blood groups in human blood, with the aid of natural isoagglutinins is

⁴² *Jour Immunol* 18: 87 (1930)

⁴³ Wiener, *Amer Jour. Clin Path* 12: 302 (1942)

not very practicable for two main reasons. Firstly, sera containing irregular isoagglutinins are rare, and of those with the highest incidence only anti P is useful, because agglutinins for A₁ and O are more readily obtained by other methods. Secondly, irregular isoagglutinins are weak, as a rule, and may deteriorate quickly.

Immune Isoagglutinins—As has been pointed out particularly by Wiener,⁴⁵ the occurrence of isoimmunization in man can be demonstrated most strikingly by tests on sera from patients who have been transfused unintentionally with blood of an incompatible group (cf page 64). It appears from these observations and from the report of Biancalana and Teneff⁴⁶ that the agglutinogens A and B are good antigens in man for those individuals who lack the corresponding agglutinin. The almost completely negative results obtained by Thomsen⁴⁷ can most likely be ascribed to the small doses he injected.

These results and the evidence that has been collected for the existence of a large number of individual differences in human blood might lead one to believe that isoimmunization would be an effective method also of detecting intragroup differences, and this view would seem to be supported by some of the results obtained in lower animals (cf Chapter XIX). However, the paucity of positive information on this subject until recent years tends to contradict this view. The reason why isoimmunization yields so few positive results is that the agglutinogens responsible for intragroup differences are either non antigenic or only poor antigens in man. For example, M and N are extremely poor antigens in man⁴⁸ even though they are good antigens for rabbits and other animals (cf page 118).

The first deliberate search for immune isoagglutinins formed as a result of transfusion was made by Landsteiner, Levine and Janes,⁴⁹ who examined the sera of 7 individuals who had been repeatedly transfused with blood of their own group. In 5 of these sera no abnormal agglutinins were noted. In the sixth case, an abnormal agglutinin appeared in the recipient's serum after the first and second transfusion but it was later noticed that the patient's serum had contained a very weak irregular isoagglutinin before the transfusions were given. In the serum of the seventh patient, who belonged to group A, the presence

* Cf review by Hooker [*New Engl Jour of Med* 225: 871 (1941)].

⁴⁵ Wiener *Blood Groups and Blood Transfusion* p. 98 2nd edition (1939).

Wiener, Oremland, Hyman and Samwick *Amer Jour Clin Path* 11: 102 (1941).

Wiener *Jour Immunol* 41: 181 (1941) cf *Ro Acto chir Scand* 80: 283 (1937).

⁴⁶ *Boll Soc Int Micro Sc Ital* 2: 397 (1930).

⁴⁷ *Ztschr f Rassenphysiol* 2: 105 (1930).

⁴⁸ Wiener and Forer *Proc Soc Exp Biol and Med* 47: 215 (1941) Wiener *Amer Jour Clin Path* 12: 302 (1942).

⁴⁹ *Proc Soc Exp Biol and Med* 25: 672 (1928).

of weak isoagglutinins acting on the blood of group O and subgroup A₂ was detected. Accordingly, in the latter two cases, there may have been an isoimmunization effect.

The present author undertook a similar investigation of post transfusion sera several years ago but abandoned the project when no positive results were obtained. The reason for the negative findings becomes clear when we consider that by the method of differential agglutination⁵⁰ it has been shown that the donor's blood cells survive in the patient's circulation for periods up to three or four months (cf. page 73), while the appearance of immune isoagglutinins in the patient's serum would hardly be expected until all the donor's cells have been eliminated. Apparently individuals having the capacity to eliminate blood of the homologous group rapidly from their bodies and to form immune isoantibodies are rare. Accordingly, sera containing immune isoagglutinins are generally found either (1) by chance in cross matching tests in patients receiving repeated transfusions or (2) by the investigation of selected sera, namely, from patients with hemolytic transfusion reactions (cf. page 117).

As an example, the case reported by Neter⁵¹ may be cited. He observed the appearance in a patient's serum of immune isoantibodies following a blood transfusion, when the matching tests were repeated preliminary to a second transfusion from the same donor. In tests made at 37°C the patient's serum when fresh hemolyzed and when inactivated agglutinated, certain other bloods of compatible groups. At low temperature, only weak agglutination was obtained; the serum in contrast to those containing natural isoagglutinins reacting most intensely at higher temperatures. The present author⁵² has examined 6 sera from patients receiving repeated transfusions which were referred to him because in the matching tests they were found to contain irregular isoagglutinins. The immune isoagglutinins in 2 cases proved to be anti P, one each were anti A₁, anti O, anti M and anti Rh.

In cases of hemolytic transfusion reactions a number of investigators have detected irregular isoagglutinins or isohemolysins in the patients' sera.⁵³ Examining such sera, Wiener and Peters,⁵⁴ as mentioned before, observed that the great majority contained isoantibodies corresponding

⁵⁰ Wiener *Amer Jour Clin Path* 12: 189 (1942).

⁵¹ *Jour Immunol* 30: 255 (1936).

⁵² Wiener et al. *Amer Jour Clin Path* 11: 102 (1941). Wiener *Amer Jour Clin Path* 12: 302 (1942).

⁵³ Lach *Ztschr f Rassenphysiol* 8: 1 (1936). Culbertson and Ratcliffe *Amer Jour Med Sci* 192: 471 (1936). Pondman *Nederl Tijdschr von Geneesk* 82: 6111 (1938), etc.

⁵⁴ Wiener and Peters *Ann Int Med* 13: 2306 (1940).

to the property Rh, and in the examination of one of the sera, the existence of subdivisions of the Rh type was established⁵ (cf page 252)

Because of the rarity of intragroup isoimmunization by transfusion, the study of individual blood differences by this method is difficult. A step forward was made when it was discovered that isoimmunization can also result from pregnancy. In titration experiments on the sera from post partum patients, Jonsson⁵⁴ observed that in group O mothers bearing group A children, the average titer of the anti A isoagglutinins is distinctly higher than that of the anti B isoagglutinins, while in group O mothers bearing group B infants the situation is reversed. These findings suggest the passage of fetal blood or plasma containing group substances (cf page 117) into the maternal circulation with resulting isoimmunization. At about that time (1935), the present author⁵⁷ encountered a post partum patient of subgroup A with exceptionally potent α_1 agglutinins in her serum. While at first these agglutinins had been believed to be natural irregular isoagglutinins, after Jonsson's report the possibility had to be considered that they might be the result of isoimmunization to the stillborn infant's blood (presumably of subgroup A₁). This idea received support from Levine and Stetson's⁵⁸ observation of a hemolytic transfusion reaction which they traced to isoimmunization of the mother by the fetus. Furthermore, in an analysis of intragroup hemolytic transfusion reactions, Wiener and Peters⁵⁹ noted that every such reaction to a first transfusion occurred in a post partum patient. Nevertheless, isoimmunization from pregnancy is not a common occurrence, so that in the examination of random post partum sera irregular isoagglutinins will not as a rule be encountered. Presumably two factors are necessary in order for this phenomenon to occur: a defect in the placenta, permitting the passage of fetal blood into the maternal circulation, and a patient capable of responding to the introduction of fetal blood by the production of isoantibodies.

The detection of post partum sera containing immune isoagglutinins was facilitated when Levine, Katzin and Burnham⁶⁰ discovered the relationship between isoimmunization in pregnancy and the occurrence of erythroblastosis fetalis (cf page 569). By studying sera from mothers of erythroblastotic infants, immune irregular isoagglutinins are demonstrable in at least half the cases.⁶¹ Here again the most com

⁵⁴ Wiener *Arch Path* 32: 227 (1941)

⁵⁵ *Acta Pathol et Microbiol Scand* 13: 424 (1936)

⁵⁶ *Jour Immunol* 41: 181 (1941)

⁵⁷ *Jour Amer Med Assoc* 113: 126 (1939)

⁵⁸ *Ann Int Med* 13: 2306 (1940)

⁵⁹ *Jour Amer Med Assoc* 116: 825 (1941)

⁶⁰ Levine, Burnham, Katzin and Vogel *Amer Jour Obst and Gyn* 42: 925 (1941) Wiener *Amer Jour Clin Path* 12: 302 (1942)

mon irregular isoagglutinin proved to be anti Rh, and indeed as already mentioned (cf page 246), some of these sera are the most potent reagents for testing for the Rh factor. There is evidence that still further individual differences in human blood may be demonstrable by this method. (For example, see the report by Levine and Polayes⁶²)

In conclusion, the important role that the constitution of the individual plays in the capacity to form immune isoantibodies should be pointed out again. In this connection, it is of interest that in a relatively short period, the present author⁶³ has observed two cases of multiple isoimmunization, which would hardly be expected if isoimmunization occurred at random.

INDIVIDUALITY OF THE SERUM⁶⁴

The antibodies which have been found in human and animal sera have been classified as normal (or natural) and immune. The latter whether acquired as a result of manifest or unapparent infection or resulting from the parenteral injection of foreign proteins, bacteria, blood cells, etc., determine individual blood differences though not of constitutional nature.

There are certain normal antibodies which appear to be to some extent characteristic of the species. As an example may be cited Landsteiner's observations (cf page 30) on the occurrence of anti A and anti B agglutinins in the sera of various monkey species. However in general there exist variations among the sera of individuals of the same species with regard to their content of normal agglutinins due to constitutional differences. This is the main reason why it is so difficult to prepare reagents from normal animal sera for demonstrating agglutinable properties in human blood other than A, B or O (cf page 262). As an example may be cited the results of an experiment made by the author with normal cat sera. Different cat sera were studied and it was found that 2 gave little or no agglutination reactions in tests with human red cells, one serum contained anti A agglutinins and another anti O agglutinins but with little or no human species agglutinins, the fifth contained moderately potent species agglutinins and in addition anti A, anti B and anti O agglutinins while the remaining two sera contained potent human species agglutinins, moderately strong anti A, anti B and anti-O agglutinins and besides one or two weak hetero-agglutinins defining individual differences in human blood independent of A and B (cf page 263).

Several studies indicate the hereditary nature of normal antibodies. Thus Landsteiner and Levine⁶⁵ found that the incidence of anti P agglutinins in the offspring of rabbits possessing such agglutinins is much higher than in adult rabbits taken at random. And Stuart et al.⁶⁶ demonstrated that the presence of normal anti A agglu-

⁶² *Ann Int Med* 14: 1903 (1941)

⁶³ Wiener and Forer, *Proc Soc Exp Biol and Med* 47: 245 (1941); Wiener, Silverman and Aronson, *Am Jour Clin Path* 12: 241 (1942)

⁶⁴ Cf Landsteiner, *Specificity of Serological Reactions* p. 86 (1936)

⁶⁵ *Jour Immunol* 20: 149 (1931)

⁶⁶ Stuart, Sawin, Wheeler and Battey, *Jour Immunol* 31: 25 (1936)

tinin in rabbit sera is inherited, this property probably being transmitted as a simple Mendelian recessive.⁶⁷

In man, certain normal antibodies (for example, hemagglutinins for rabbit blood) are present in practically every serum, whereas others are characteristic of the individual, such as the common isoagglutinins α and β . The presence of an anomalous isoagglutinin in a person's serum may serve to differentiate him from other individuals, but because of their rarity they can only exceptionally be used for individual identification. According to observations made by Nigg⁶⁸ on extra-agglutinin 1 in one family, it seems not unlikely that this agglutinin may have an independent heredity as a Mendelian dominant.

Heteroagglutinins in human serum for blood of certain species show considerable individual variation.⁶⁹ This phase of the problem of the individualization of the blood merits further investigation.

⁶⁷ Also, see Kaempffer *Ztschr f indukt Abstamm u Vererbungs* 61. 261 (1932)

⁶⁸ *Jour Immunol* 19. 1 (1930)

⁶⁹ Cf. Sievers, *Acta Path et Microbiol Scand* 14. 553 (1937)

CHAPTER XVII

GROUP SPECIFIC SUBSTANCES IN ORGANS AND BODY FLUIDS, STUDIES ON THEIR CHEMICAL NATURE

THE DIVISION of human beings into four groups O A B and AB is not limited to the blood since group specific substances are present in the cells of almost every organ of the body and in most individuals (secretors) also in the body fluids and secretions With respect to factor A there are also demonstrable differences in the tissues and body fluids corresponding to the two subgroups¹

Group Specific Substances A and B in Organ Cells Landsteiner and Levine² demonstrated for the first time the presence in man of the group specific substances A and B in cells other than the erythrocytes They showed that spermatozoa³ have the capacity of specifically absorbing group agglutinins from immune serum Previously von Dungern and Hirschfeld⁴ had observed the existence of substances in the kidneys of dogs specific for individuals

Kritschewski and Schwarzmann,⁵ Witebsky,⁶ and Witebsky and Okabe⁷ stated that they could show, by using various technics that the organ cells have the same group properties as the blood Kritschewski and Schwarzmann found that suspensions of organ cells (spleen liver kidney, etc) that had been freed of as much blood as possible had the power to specifically absorb the isoagglutinins Witebsky and Okabe demonstrated the presence of the A factor in alcoholic extracts of the organs of individuals of group A by means of complement fixation reactions with anti A immune rabbit sera

According to Semzowa and Terechova,⁸ group-specific substances can be demonstrated in the cells of the organs of fetuses after the sixth month

¹Thomsen [*Acta Pathol et Microbiol Scand* 7 258 (1930)] showed that in general the tissues of individuals of subgroup A_2 have a lesser capacity to bind agglutinins than the tissues of individuals of subgroup A_1

²*Jour Immunol* 12 415 (1926)

³At about the same time Yamakami [*Jour Immunol* 12 185 (1926)] demonstrated the presence of group-specific substances in the spermatic fluid.

⁴*Ztschr f Immunstats* 8 554 (1910-11)

⁵*Alin Hoch* 6 2090 (1927) 7 896 (1928)

⁶*Alin Hoch* 7 118 (1928) *Ztschr f Immunstats* 49 1 517 (1926-27)

⁷*Ztschr f Immunstats* 52 359 (1927)

⁸*Ukr Zentralbl f Blutgruppenforsch* 2 130 (1929) *Alin Hoch* p 206 (1929)

Whether the group-specific substances are present in the placenta is a question of special interest. V. Ottingen and Witebsky⁹ failed to find any specific substances in alcoholic extracts of this organ. Subsequent workers using varying techniques, obtained similar results. Reich¹⁰ made a particularly careful study of this problem, by separating the various parts of the placenta. He found that the amnion contained the group-specific substances of the child, whereas the decidua contained the group-specific substances of the mother,¹¹ the decidua parietalis being much richer in group-specific substances than the decidua basalis. The chorion frondosum, on the other hand, contains no specific substances, although richest in blood, and thus constitutes a "neutral zone." The absence of group specific substance from the placental tissues has been explained by Schiff and Weiler¹² as being due to the presence of a blood group enzyme capable of destroying the substances A and B (cf. page 286).

The presence of group specific substances is not a property restricted to normal tissues, for, as Thomsen¹³ has shown, the cells of tumors, both benign and malignant, have the capacity of specifically absorbing agglutinins.

Direct agglutination of cells other than the erythrocytes has not been accomplished with the possible exception of leucocytes. Thus Wichels and Lampe¹⁴ reported that suspensions of leucocytes from two cases of leukemia were agglutinated like the red blood cells by human anti A serum. On the other hand, in a study of the leucocytes from fourteen cases of leukemia, Thomsen¹⁵ found that direct agglutination of these cells could be accomplished only exceptionally, although the cells absorbed specifically agglutinins against the factors A and B.

In most of the earlier studies, the presence of group-specific substances in the organ cells was demonstrated by the absorption technique (cf. page 26). A few workers, especially Witebsky and his collaborators, employed the complement fixation technique by testing alcoholic extracts of the tissues with immune rabbit sera produced by injections of human blood. This method was applied only to the group-specific substance A, however, because rabbits seldom produce anti B sera of high enough titer for such tests (cf. page 286). As will be pointed out later in most individuals (secretors) group specific substances are present in the organs in a water soluble form and can be demonstrated in isotonic aqueous extracts by the "inhibition" technique.

The relative quantities of group-specific substances in different or-

⁹ *Munch med Woch* 75: 385 (1928)

¹⁰ *Ztschr f Immunitäts* 77: 449 (1932)

¹¹ Rarely the decidua parietalis contained the group-specific substances of the child which probably had reached it by diffusion.

¹² *Biochem Zeitschr* 235: 454 (1931)

¹³ *Loc cit*, cf. Hirsfeld Halber, and La-kowski, *Ztschr f Immunitäts* 64: 61 (1929)

¹⁴ *Klin Woch* 7: 1741 (1928)

¹⁵ *Acta Pathol et Microbiol Scand* 7: 250 (1930)

gans have been determined by Hirszfeld,¹⁶ Schiff⁷ and Friedenreich and Hartmann.¹⁸ The largest quantities were found in stomach, submaxillary gland and pancreas; moderate amounts in kidney, parotid gland, liver, prostate, lungs, spleen and myocardium; smaller amounts in the large intestines and only traces in the testes. In the brain the small quantities found are perhaps due to contamination with blood (Schiff). Group specific substances seem to be absent from the lens of the eye, hair, compact bone, cartilage and skin epithelium.¹⁹

Group Specific Substances in Body Fluids and Secretions Moss²⁰ and Grafe and Graham²¹ showed that the blood serum contains substances capable of specifically inhibiting the effect of isohemolysins present in fresh normal human sera. e.g., the serum of an individual of group A can prevent the hemolysis of erythrocytes of group A by fresh human sera containing the isohemolysin anti A. This phenomenon was attributed by these authors to the presence in the serum of anti hemolysins. Schiff²² demonstrated however that the serum contains group specific substances in solution and the observations of the earlier workers can be explained more reasonably by the inhibiting effect of such antigens rather than by the action of hypothetical anti antibodies. Schiff obtained an anti A immune rabbit serum which gave precipitation reactions with the sera of individuals of group A and group AB but not with those of individuals of groups O and B. Yamakami²³ demonstrated the presence of group specific substances in spermatic fluid and in the saliva of one individual by means of the inhibition technique. Shortly thereafter as a result of studies made by Lehrs²⁴, Puthonen,²⁵ Yosida,²⁶ Schiff²⁷, Thomsen²⁸ and others, group specific substances were found in tears, sweat, urine, digestive juices, bile, milk, pleural, pericardial and peritoneal fluids and amniotic fluid and also in the fluids of hydro-

¹⁶ Hirszfeld, Halber and Laskowski, *Klin. Woch.* 8, 1563 (1929).

¹⁷ *Loc. cit.*

¹⁸ *Ztschr. f. Immunitäts* 92, 141 (1938).

¹⁹ Yosida, *Ztschr. f. d. ges. exp. Med.* 63, 331 (1928); Kritschewsky and Schapiro, *Ztschr. f. Immunitäts* 59, 264 (1929).

²⁰ *Bull. Johns Hopkins Hosp.* 21, 63 (1910); *Folia serol.* 5, 26 (1910).

²¹ *Monatsh. med. Woch.* 58, 115, 2338 (1911).

²² *Klin. Woch.* 3, 16 (1924).

²³ *Jour. Immunol.* 12, 185 (1926).

²⁴ *Ztschr. f. Immunitäts* 66, 15 (1930).

²⁵ *Acta Med. Fenn. Diodecim.* A 14, Part 2 (1930).

²⁶ *Ztschr. f. d. ges. exp. Med.* 63, 331 (1928).

²⁷ *Über die gruppenspezifischen Substanzen des menschlichen Körpers*, Jena (1931).

²⁸ *Loc. cit.*

celes and ovarian cysts. On the other hand, these substances could not be demonstrated in the cerebrospinal fluid.

Table 60 gives the approximate relative concentration of group-specific substances in various body fluids and secretions and in the erythrocytes. The high concentration of group-specific substances in such secretions as saliva, semen, gastric and duodenal juices, etc., is explained by the large quantities present in the cells of glandular tissue.

TABLE 60
RELATIVE CONCENTRATION OF GROUP SPECIFIC SUBSTANCES IN
VARIOUS BODY FLUIDS
(After Putkonen)

Saliva	128-1024
Semen	128-1024
Amniotic Fluid	64-256
Erythrocytes	8-32
Tears	2-8
Urine	2-4
Cerebrospinal Fluid	0

In this connection, Hirszfeld²⁹ states that he could not demonstrate the presence of group specific substances in the testes, but found them to be present in high concentration in the prostate and he believes that the prostatic secretion is the source of the group-specific substances in seminal fluid.

SECRETORS AND NON-SECRETORS

In the first studies on the presence of group-specific substances in saliva, variations were noted between different individuals and also in the same individual from time to time. Subsequent studies have revealed that the variations noted in the saliva of one and the same individual are due largely to the action of a blood-group enzyme present in normal saliva (cf. page 286). On the other hand, Lehrs³⁰ and Putkonen³¹ were the first to establish the striking fact that the saliva of certain individuals of groups A, B and AB contain the corresponding group-specific substances in concentrations much higher than the red blood cells, whereas such substances are practically lacking in the saliva of other individuals of the same blood groups. Schiff and Sasaki³² have designated individuals of the former type "secretors;" those of the latter type are called "non-secretors." These authors have shown that group

²⁹ Cited after Friedenreich [*Ztschr f Immunitäts* 91: 1 (1937)]

³⁰ *Ztschr f Immunitäts* 66: 175 (1930)

³¹ *Acta Med Fenn "Duodecim,"* A 14, Part 2 (1930)

³² *Ztschr f Immunitäts* 77: 129 (1932), *Klin W'och* 11: 1426 (1932)

O individuals can be subdivided in a similar manner by testing their saliva with anti O sera

The ability or inability to secrete group specific substances in the saliva is a constant trait of the individual. Moreover Putkonen³² and Sasaki³⁴ found that when group-specific substances are lacking in the saliva of an individual, they are also absent from the other secretions, such as urine, tears, semen, gastric juice and milk.

The nature of the difference between secretors and non secretors has been clarified by the study of organ extracts. Schiff³ succeeded in extracting the group specific substances in two forms one soluble in organic solvents such as alcohol and chloroform the other soluble in aqueous solutions. According to Friedenreich and Hartmann¹⁶ these two forms of the group substances designated as lipoidal and water soluble respectively, give rise to two systems and differences among individuals with regard to the water soluble group substances are responsible for the existence of the secretor and non secretor types of Schiff and Sasaki. In the erythrocytes and almost all organs of all individuals whether secretors or non secretors the lipoidal form of the group substances is present. On the other hand water soluble group substances cannot be readily obtained from erythrocytes though they can be easily extracted from the tissues of secretors being lacking from or present in only small amounts in aqueous organ extracts of non secretors. The quantities of the water soluble group substances in secretors are highest in the glandular organs the concentration in different glands paralleling the concentration in the corresponding secretions (cf figure 57 and table 60) indicating that the group substances in secretions are formed in the respective glandular organs and are not derived from the blood.

As an example of the independence of the two systems of group substances Friedenreich³⁷ has pointed to the conditions in lower animals. In some species, such as horses, guinea pigs and rabbits the presence of substances similar to the group substances A and B can be demonstrated in high concentration in the secretions and organs while corresponding group substances are entirely lacking from the erythrocytes. On the other hand, substances related to the group substance A are present in sheep erythrocytes though lacking from sheep organs and secretions.

³² *Loc cit*

³⁴ *Ztschr f Immunitäts* 77 101 (1932)

³⁷ *Ueber die gruppen-spezifischen Substanzen des menschlichen Körpers* Gustav Fischer Jena (1931)

³⁸ *Ztschr f Immunitäts* 92 141 (1938)

³⁹ *Ztschr f Immunitäts* 91 39 (1937)

Direct evidence that the two systems of group-substances are distinct has been obtained by comparing the serological reactions of the group substance A in erythrocytes and secretions. Thus, group A saliva reacts with rabbit immune sera for group A blood less intensely than group A erythrocytes, although saliva reacts better than blood with natural human anti-A sera³⁵

Heridity of the Secreting Type. The hereditary nature of the characters secretor and non-secretor, was first demonstrated by Schiff and Sasaki

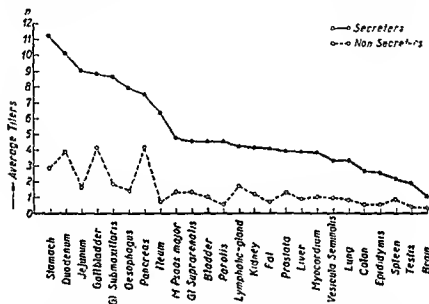


FIG 57 AVERAGE ANTIGEN CONCENTRATION IN 25 ORGANS OF THE HUMAN BODY

(Reproduced from Hartmann, *Group Antigens in Human Organs* 1941)

The aqueous extracts used in the tests were all prepared by the identical method from equal amounts of tissue. The titers are expressed as powers of 2, so that the value 11 represents titer 2^{11} or about 2000

In a series of 50 families with 149 children it was found that the ability to secrete group-specific substances was transmitted as a simple Mendelian dominant. In the few additional studies subsequently made, there has been no exception to the dominance rule (cf table 61). In the families tested by Schiff and Sasaki, the blood of each individual was also tested for the agglutinogens A, B, M and N, but no evidence was found of linkage between the character "secretor" and any of the agglutinogens

³⁵ Friedenreich, Thyssen and Hartmann, *Jour Immunol* 37: 435 (1939), Wiener and Kosofsky, *Jour Immunol* 42: 381 (1941)

Because of their hereditary nature, the characters *S* and *s* may find application in forensic medicine in paternity disputes (cf page 391) Moreover, the traits, *S* and *s*, double the number of serological classes of human beings so that, together with the characters *A*₁, *A*₂, *B*, *M*, *N* and two varieties each of *Rh* and *P*, 576 types of individuals are distinguishable

TECHNIC

For distinguishing secretors from non secretors the most suitable material is saliva It is easy to obtain and permits of a sharp distinction between the two types The saliva is placed at once in boiling water for 10 to 20 minutes in order to inactivate any enzymes present (cf page 286) The coagulum is thrown down by centrifugation and the supernatant slightly opalescent fluid pipetted off This boiled saliva can be stored in the refrigerator for a long time without any appreciable change in activity

The author collects saliva from newborn infants and very young children as follows A thick cotton swab is rubbed over the floor of the mouth until the cotton is saturated The wet cotton swab is then forced into a narrow tube in order to squeeze out the saliva This process is repeated using the same swab until enough material (about 5 drops or more) is obtained for the tests

For testing saliva of groups *A*, *B* and *AB* human group *A* and group *B* sera are preferable to immune rabbit sera for the reason already pointed out (cf page 271) For the tests the serum is diluted so as to contain about 8 units of reagglutinin per unit volume Thus a serum with a titer of 64 (cf page 17) would be diluted with about 7 volumes of saline solution before use As a rule better results will be obtained by using high titered sera properly diluted than with low titered undiluted sera For uniform results in the tests for the group-substance *A* it is necessary to

TABLE 61
HEREDITY OF THE SECRETING TYPE

Parental Combination	Number of Families	Number of Children		
		<i>S</i>	<i>s</i>	Totals
<i>S</i> × <i>S</i>	105	241	33	274
<i>S</i> × <i>s</i>	62	103	67	170
<i>s</i> × <i>s</i>	18	0	42	42
Totals	185	344	142	486

S = Secretor *s* = Non secretor

This table includes

50 families with 149 children by Schiff and Sasaki (*loc cit*)

44 families with 115 children by Morzycki [*Compt rend Soc de Biol* 115 658 (1934)]

32 families with 89 children by Matson and Brady [*Jour Immunol* 40 445 (1936)]

18 families with 53 children by Zieve Wiener and Fries [*Imm Eug* 7 163 (1936)]

and 41 families with 80 children by Suzuki [*Juzenkai Zasshi* (Japanese) 41 2594 (1936)]

use consistently standard cells of a single subgroup in the author's experience somewhat preferably A₁.

As has already been mentioned group O individuals can be classified as secretors and non secretors with the aid of anti O sera. Such reagents are obtained most easily by absorbing selected ox sera with A₁B cells. Anti O sera are inhibited not only by the saliva from group O secretors but also by saliva from secretors of groups A, B and AB.³⁹ However the distinction between the two types secretor and non secretor is not as sharp with anti O sera as with group A and group B sera. The probable reason for this is that the anti O sera obtainable are almost invariably of low titer. A special anti O serum obtainable only with great difficulty, namely Eisler's anti dysentery (Shigae) immune goat serum (cf. page 338) was found by Schiff⁴⁰ to be usable in high dilution and to give a sharp distinction between secretors and non secretors of group O.

The actual tests can be carried out either qualitatively or quantitatively. The results are more readily controlled when the tests are made quantitatively so that this procedure should always be followed in forensic cases. However reliable results can also be obtained as a rule by the simpler qualitative procedure which will be found satisfactory for less important routine examinations.

Qualitative Tests

Inasmuch as saliva from non secretors gives weak inhibition reactions when tested in high concentration the saliva should be sufficiently diluted for the tests. For saliva of groups A, B and AB dilution 1:10 will be found to give satisfactory results in tests with anti O sera. dilution 1:5 is preferable.

The tests for saliva of groups A, B or AB are set up as follows:

Tube (1) 1 drop diluted saliva + 1 drop diluted A serum

Tube (2) 1 drop diluted saliva + 1 drop diluted B serum

Tube (3) 1 drop saline + 1 drop diluted A serum

Tube (4) 1 drop saline + 1 drop diluted B serum

After these mixtures have stood for 10 minutes or longer at room temperature 1 drop of standard A₂ blood suspension is added to tubes (2) and (4) and 1 drop of B blood suspension to tubes (1) and (3). The reactions are read after the mixtures have stood an hour at room temperature. Tubes (3) and (4) are controls and should show agglutination. If agglutination occurs in tube (1) but not in tube (2) the saliva contains group-substance A; agglutination in tube (2) and not in tube (1) occurs with saliva from a group B secretor; absence of agglutination from both tubes (1) and (2) indicates that the individual is a group AB secretor. Agglutination occurs in both tubes (1) and (2) when the individual belongs to group O or is a non secretor. The tests to determine whether group O individuals are secretors or non secretors are set up in a similar manner using anti O sera and test cells of group O.

For best results with every set up samples of standard saliva should be included as controls.

Quantitative Tests

With the quantitative method of examining secretions the results are more reliable. While this technic shares all the limitations of serological titrations in general if it is carefully carried out consistent results can be obtained. Thus relatively little variation is observed in the concentration of group-substances in the

³⁹ Cf. Witelsky and Klend-boj *Jour. Exp. Med.* 73: 655 (1941).

⁴⁰ *Ztschr. f. Immunitats* 82: 302 (1934).

TABLE 63

COMPARISON OF THE INHIBITIVE TITER OF THE SALIVA AND THE ABSORPTIVE TITER OF THE BLOOD OF A GROUP B INDIVIDUAL, USING DIFFERENT ANTI B SERA

Units of Agglutinin in Serum (or In Serum Dilution)	Human Serum #1			Human Serum #2			Human Serum #3			Human Serum #4			Rabbit Immune Anti B Testing Fluid		
	Serum dilution	Inhibitive titer of saliva	Absorptive titer of blood cells	Serum dilution	Inhibitive titer of saliva	Absorptive titer of blood cells	Serum dilution	Inhibitive titer of saliva	Absorptive titer of blood cells	Serum dilution	Inhibitive titer of saliva	Absorptive titer of blood cells	Serum dilution	Inhibitive titer of saliva	Absorptive titer of blood cells
128	Undiluted	2 ⁴	2 ¹	Undiluted	0	0	Undiluted	0	0	Undiluted	0	0	Undiluted	0	0
64	1 2	2 ⁶	2 ¹	1 2	0	0	1 2	0	0	1 2	0	0	1 2	0	0
32	1 4	2 ⁸	2 ²	1 4	2 ¹	2 ⁰	1 4	2 ¹	2 ⁰	1 4	2 ¹	2 ⁰	1 4	2 ¹	2 ⁰
16	1 8	2 ¹⁰	2 ³	1 8	2 ³	2 ¹	1 8	2 ³	2 ¹	1 8	2 ³	2 ¹	1 8	2 ³	2 ¹
8	1 16	2 ¹²	2 ⁴	1 16	2 ⁵	2 ³	1 16	2 ⁵	2 ³	1 16	2 ⁵	2 ³	1 16	2 ⁵	2 ³
4	1 32	2 ¹⁴	2 ¹⁰	1 32	2 ⁷	2 ⁴	1 32	2 ⁷	2 ⁴	1 32	2 ⁷	2 ⁴	1 32	2 ⁷	2 ⁴
2	1 64	2 ¹⁶	2 ¹¹	1 64	2 ⁸	2 ⁵	1 64	2 ⁸	2 ⁵	1 64	2 ⁸	2 ⁵	1 64	2 ⁸	2 ⁵
1	1 128	2 ¹⁸	2 ¹²	1 128	2 ⁹	2 ⁶	1 128	2 ⁹	2 ⁶	1 128	2 ⁹	2 ⁶	1 128	2 ⁹	2 ⁶

N.B. Serum #1 is a group A serum of a patient given a 1% capsaic injection of horse serum

TABLE 64

PROTOCOL ILLUSTRATING DIAGNOSIS OF SECRETORS AND NON SECRETORS BY QUANTITATIVE TECHNIC

Saliva Num ber	Blood Group	Saliva Dilution+Group A Test Serum +Test Cells B										Saliva Dilution+Group B Test Serum+Test Cells A ₂										Inhibitive Titer for		Diagnosis
		2 ⁰	2 ¹	2 ²	2 ⁴	2 ⁶	2 ⁸	2 ¹⁰	2 ¹²	2 ¹⁴	2 ⁰	2 ¹	2 ²	2 ⁴	2 ⁶	2 ⁸	2 ¹⁰	2 ¹²	2 ¹⁴	A	B			
		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
1	Standard A Saliva	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2 ¹²	0	—	
2	Standard B Saliva	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	2 ¹¹	—	
3	A ₁	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2 ¹¹	0	Secretor	
4	A ₁	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	Non secretor	
5	A ₁ B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2 ¹¹	2 ¹¹	Secretor	
6	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	2 ¹²	Secretor	
7	A ₂	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2 ¹¹	0	Secretor	
8	A ₂ B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2 ¹²	2 ¹²	Secretor	
9	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	2 ¹	Non secretor	
10	A ₁ B	tr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	Non secretor	

saliva differ markedly. This is probably due mainly to the differences between the technic used for blood and for saliva absorption and inhibition respectively and in part to the difference in quality between the group-substances in blood and saliva. Accordingly the amounts of group-substance in blood in saliva cannot be compared directly. Table 63 also demonstrates the superiority of high titered sera such as serum #1 over sera of low titer. Thus while serum #3 gives almost normal titers when used in dilution 1:4 (corresponding to only 2 units of agglutinin per unit volume) the use of such sera is inadvisable because the inhibitive titer increases so much more rapidly than the serum dilution that a relatively small error in diluting the serum could cause a gross error in the titer. Apparently some sera are unsuitable because the agglutinins are not readily absorbable e.g. serum #2 which came from a patient who had developed potent isoantibodies as a result of injections of horse serum. After a suitable serum has been standardized it can be used with uniform results for several years since the sera remain stable for a long time when stored in the refrigerator.

The actual tests are set up by mixing a series of dilutions of the saliva with the selected dilution of test serum and then adding the test cell suspension. For ordinary purposes a series of doubled dilutions is too cumbersome while dilutions by powers of 10 as recommended by some workers are too widely spaced. In the author's experience a series of eight dilutions by powers of 4 gives satisfactory results and by interpolation the end point can be obtained to the nearest power of 2. For making the saliva dilutions graduated pipettes can be used but this method is cumbersome. The dilutions are most readily made by counting out drops with the aid of a capillary pipette and nipple but unless great care is used in rinsing the pipette false high titers will be obtained. The following technique has proved satisfactory in the author's hands.

A rack containing three rows with 8 tubes in each row is used. The first row S for the saliva dilutions, the second row A for the diluted test serum A and the third row B for the diluted test serum B. In the first tube of row S is placed the saliva to be tested and the remainder of the tubes in the row are filled with saline. One starts with the second tube of row S by removing the saline with the capillary pipette but from the last pipetteful 9 drops are counted back into tube S₂ the remainder being discarded. Then with the same pipette 3 drops of the undiluted saliva are transferred from tube S₁ to tube S₂ to make dilution 1:4 and 1 drop of undiluted saliva is placed in the first tubes of the second and third rows (A₁ and B₁). The pipette is rinsed by removing the saline from tube S₃ but before discarding the last pipetteful 9 drops of saline are counted back into the tube. Then 3 drops of saliva dilution 1:4 are transferred from tube S₂ to S₃ to make saliva dilution 1:16 and at the same time 1 drop each of saliva dilution 1:4 is placed in tubes A₂ and B₂. This process is repeated until rows A and B both contain the complete series of dilutions of saliva. (The method outlined gives accurate results because the pipette is rinsed repeatedly with fresh saline rather than with saline from a common reservoir.) To row A 1 drop of the diluted group A test serum is added and to row B 1 drop of the diluted B serum. After 10 minutes or longer 1 drop of test cells B is added to each tube in row A and 1 drop of test cells A₂ is added to row B. Readings are taken after these mixtures have stood for one hour at room temperature. A sample protocol is given in table 64.

By the quantitative technique outlined it is possible to compare the concentration of group substances in different secretions. This has been done by Friedenreich and Hartmann⁴² who obtained complete inhibition with dilutions of gastric juice up to

⁴² *Ztschr. f. Immunitäts* 92: 141 (1933)

1 2¹⁶ saliva diluted 1 2¹¹, semen 1 2⁸, bile 1 2⁹ urine 1 2, while blood serum gave only partial inhibition even when tested undiluted. The titers just given are average values of course since samples of the same secretion from different individuals give different titers though all the values fall within a narrow range. It should be mentioned that with gastric juice which gives the highest titers significant inhibition (titers up to 2⁴) are also obtained with material from non secretors.

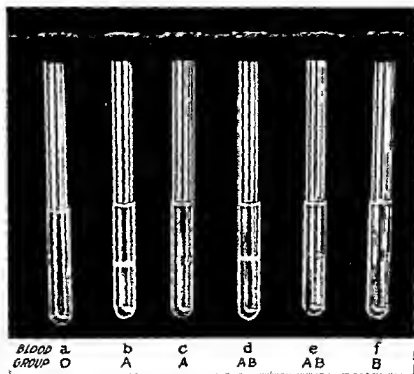


FIG 58 DEMONSTRATION OF PRESENCE OF A SUBSTANCE IN SALIVA BY PRECIPITATION TECHNIC

(After Schiff)

Saliva from individuals of various blood groups (diluted 1 100) were layered over immune serum anti A. White rings were formed with saliva specimens b and d containing property A ("secretors"). Ring formation is absent not only with saliva of group O and group B but also with specimens of non secretors (c and e).

An alternative method of differentiating secretors and non secretors is to mix the undiluted testing serum directly with an equal volume of the undiluted boiled saliva and then to compare the titer of the serum treated in this way with that of the serum mixed with an equal volume of saline. This was the method used by the earlier investigators. The method while simpler does not differentiate secretors and non secretors as sharply.

Precipitin Test for Group-Substances As has already been mentioned Schiff succeeded in demonstrating the presence of the group substance A in secretions and

body fluids from secretors of groups A and AB by direct precipitation. For this purpose it is necessary to have available potent anti A immune rabbit sera. These sera are used undiluted without preliminary absorption to remove the species agglutinins. In the tests the boiled saliva in suitable dilution e.g. 1:10 or other secretion is carefully layered over the immune serum preferably for purposes of economy in capillary tubes. The results of such tests are illustrated in figure 58.

This technic is not suitable for general use because of the difficulty of obtaining the sera particularly sufficiently potent anti B sera. Uneyama⁴⁴ found a chicken serum containing natural anti B antibodies of exceedingly high titer that could be used for demonstrating the group-substance B by the precipitin method.

Uneyama has reported the presence in about 16 per cent of normal fowl sera of precipitins for saliva from non secretors and not reacting with saliva from secretors. Moreover Uneyama stated that by injecting rabbits with saliva from group A secretors he obtained immune sera containing A antibodies which after absorption with group A blood lost their activity for group A erythrocytes but still precipitated A secretor but not A non secretor saliva.

BLOOD GROUP ENZYMES

Schiff and Akune⁴⁵ found that solutions of group specific substances and secretions lose their group specific properties if incubated with saliva or suspensions of feces from normal human beings or lower animals due to the action of a special enzyme (Schiff and Weiler⁴⁶). Schiff's⁴ observation that some strains of gas bacillus of Frankel and Welch have the capacity to destroy blood group substances may have some bearing on the presence of the enzyme in stool filtrates.

Blood group enzymes are present in the saliva of secretors as well as non-secretors.⁴⁴ Aside from temporary changes correlated with the intake of food etc. there are distinct and persistent differences between individuals with regard to the quantity of these enzymes in the saliva. None of the known bacteria in the mouth has been implicated as the source of the blood group enzymes in saliva and Schiff and Buron⁴⁷ have reported some observations which indicate that the enzyme possibly derives from the glandular cells.

STUDIES ON THE CHEMICAL NATURE OF GROUP SPECIFIC SUBSTANCES

For studies on the chemical nature of the group substances two sorts of material are available, the substances extractable by organic solvents from the erythrocytes and organs of all human beings and the group substances in secretions and aqueous organ extracts of secretors. As has already been mentioned the two sorts of group substance differ somewhat in their serologic behavior, and this most likely would de-

⁴⁴ *Jap Jour Med Sci Part III Soc Med & Hyg* 3: 13 (1940)

⁴⁵ *Munch med Woch* p. 657 (1931)

⁴⁶ *Biochem Ztschr* 235: 454 (1931) *ibid* 239: 489 (1931) Also see Stimpl *Ztschr f Immunstats* 76: 159 (1932)

⁴⁷ *Klin Woch* 14: 750 (1935)

⁴⁸ Matson and Brady *Jour Immunol* 30: 445 (1936)

⁴⁹ *Klin Woch* 14: 710 (1935)

pend on a corresponding difference in chemical constitution. On the other hand, the group specificity shared by the two sorts of material indicates the presence in their molecules of identical or closely related structures.

The fact that the agglutinogens A and B of human erythrocytes have the power of engendering antibodies might lead one to conclude that they are protein in nature, based on the view held for a long time that only proteins can take part in immunological reactions. It is now known that the injection of substances of simple chemical structure in combination with foreign protein (Landsteiner⁵⁰) can stimulate the formation of antibodies and these are capable of reacting with the simple substances themselves. Moreover, substances of carbohydrate nature have been prepared from bacteria which not only react with the corresponding specific immune serum but under special conditions induce the formation of immune antibodies without the aid of proteins, and carbohydrate lipid complexes have been isolated which exhibit full antigenic capacity. From observations to be described presently one may assume that the antigenic power of the agglutinogens A and B as they exist in the red blood cells depends upon their association with proteins in the cells.

The first observation pertaining to the chemical nature of the group-specific substances proper was the discovery that these substances could be extracted from the red blood cells and organ cells with alcohol.⁵¹

The solubility of the group-specific substances in alcohol has been taken as evidence of their lipid nature and placed them in the same category as a group of serologically active substances commonly regarded as lipids, for example the Forssman substance and the so called Wassermann antigen. The Forssman antigen is characterized by its capacity to incite the formation of sheep cell lysins when injected into suitable animals (rabbits). It is widely distributed occurring in sheep erythrocytes, the organs of certain animals such as horses and guinea pigs, certain strains of bacteria, etc. It is of special interest in the present connection because of its relationship to the human agglutinin A (cf. page 31). Landsteiner⁵² and others found that alcoholic extracts of the Forssman antigen had the power of reacting with corresponding immune sera in flocculation and complement fixation tests although the extracts themselves had little or no antigenic power. When mixed with proteins such as human or pig serum the alcoholic extracts became antigenic. Such substances which by themselves have no full antigenic power though they have the capacity of reacting with specific immune sera have been termed haptens by Landsteiner. The group-specific substances in alcoholic extracts belonging to this category.⁵³

⁵⁰ Landsteiner, *The Specificity of Serological Reactions*, C. C. Thomas, Springfield, Ill. (1936).

⁵¹ Schiff and Adelsberger, *Zentralbl. Bakteriologie Orig.* 93, 172 (1924); Landsteiner and van der Scheer, *Jour. Exp. Med.* 41, 427 (1925).

⁵² *Biochem. Ztschr.* 93, 106 (1919); 104, 280 (1920).

⁵³ Landsteiner and van der Scheer, *loc. cit.*, Witelsky, *Ztschr. f. Immunstat.* 48, 369 (1926), *ibid.* 49, 1, 517 (1927).

The studies on the chemical nature of the Forssman antigen are of interest here because of the relationship already referred to which exists between this antigen and the agglutinin A. Landsteiner and Levene⁵⁴ succeeded in isolating highly active preparations from horse kidneys (giving complement fixation reactions with heterogenetic immune sera up to a dilution of 1 to 20 000 000) soluble in water but not in alcohol. On hydrolysis reducing substances corresponding in amount to 28 per cent as glucose and fatty acids were obtained. On account of the high carbohydrate content of their preparations and the demonstration by Heidelberger and Avery⁵⁵ and others that the specific reactions of many bacteria depend upon haptens consisting of polysaccharides Landsteiner and Levene suggested that the specific portion of the Forssman antigen was also of carbohydrate nature. Strongly in favor of this conclusion is the isolation from certain bacteria of polysaccharides with the characteristic properties of the Forssman hapten⁵⁶. The most thoroughly studied of these preparations is a carefully purified polysaccharide obtained by Morgan from Shiga dysentery bacilli⁵⁷.

The chemical nature of the Forssman hapten present in horse kidney has been studied extensively by Brunius⁵⁸. Purified extracts of this hapten did not react with Forssman antisera but were activated by the addition of non specific lipids as had been observed previously by Landsteiner and Levene⁵⁹. By suitable extraction Brunius succeeded in enhancing the purity of the hapten from 4000 to 30 000 times calculated on the basis of fresh horse kidney. The purified extracts were resistant to proteolytic enzymes gave a negative biuret reaction and on hydrolysis yielded reducing sugars and fatty acids. Brunius' findings indicate that hexosamine in acetylated form is an intrinsic constituent of the F hapten molecule. Finally inactivation of the substance by diazomethane suggests the importance of acid groupings (carboxyl) for the immunological potency of the F hapten.

Active aqueous extracts of group specific substances were obtained from human erythrocytes (also from O blood) by Hallauer,⁶⁰ after preliminary treatment of the cells or stromata with alcohol whereas on direct extraction with water only small quantities pass into solution. The preparations gave reactions for carbohydrates. Thus the group specific substances extractable from human red cells with alcohol may well be analogous in their composition to the Forssman hapten discussed above, and may contain the specific portion in combination with lipids.

The occurrence of group specific substances in water soluble form in secretions suggested their use for chemical studies. Of special advantage for this purpose because of the large quantities available are other

⁵⁴ *Jour Immunol* 14 81 (1927) *Proc Soc Exp Biol and Med* 24 693 (1927)

⁵⁵ *Jour Exp Med* 38 73 (1923) 40 301 (1924)

⁵⁶ Landsteiner and Levene *Jour Immunol* 22 75 (1932)

⁵⁷ Morgan *Biochem Jour* 30 909 (1936)

⁵⁸ Brunius *Chemical Studies on the True Forssman Hapten the Corresponding Antibody and Their Interaction* Stockholm (1936)

⁵⁹ *Jour Immunol* 14 81 (1927)

⁶⁰ *Ztschr f Immunitäts* 83 114 (1934) Cf. Kassjakow and Tribulew *Ztschr f Immunitäts* 98 26 (1940), Kassjakow, *Ztschr f Immunitäts* 99 221 (1941)

materials particularly commercial hog pepsin which have been found to be rich in A substance⁶¹ Brahn, Schiff and Weinman⁶² separated an active substance from pepsin (1/200 γ still reacted with anti A immune sera), readily soluble in water, insoluble in alcohol and containing 5.5 per cent N. On hydrolysis it yielded reducing sugars. From horse saliva and from commercial preparations of pig pepsin, Landsteiner and Chase^{3,64} isolated a highly active substance (quantities of the order 0.005 to 0.0025 γ being detectable), containing a considerable amount of glucosamine and galactose but no fatty acids.⁶⁵ From recent work of Landsteiner⁶⁶ the substance appears to contain amino acids, probably as an essential component.

In evaluating the reports just described concerning the chemical nature of the group-substances in pepsin and horse saliva as well as the more recent reports reviewed below on the group substances in human secretions some comments made by Wiener and Kosofsky⁶⁷ should be mentioned. These authors point out that the degree of purification of preparations of group substances can be measured with the aid of the quantitative inhibition test and in fact unless such control tests are made the chemical findings are of doubtful value. For example boiled saliva contains about 0.5 per cent of solid matter so that one drop as used in the tests would contain 0.25 mg. of solid of which only a small proportion is the group substance itself. Since the group-substances in saliva can easily be detected in dilutions as high as 1:2²² as little as $\frac{1}{16}\gamma$ of the impure group substance from saliva can be identified. As has already been mentioned the group-substances isolated from pig pepsin and horse saliva by Landsteiner⁶⁸ were detectable in quantities as minute as 1/2000 γ by inhibition of isoagglutination. If one can assume that the conditions in horse and human saliva are comparable then the preparations obtained by Landsteiner were roughly 200 times as pure as the solids in boiled human saliva. Incidentally by the very sensitive technique suggested by Schiff for testing for group-substance A namely inhibition of sheep cell lysis of anti A immune rabbit sera Landsteiner found a 1 per cent solution of the purified substance to be 500 times as active as the original horse saliva an increase in activity of about 250 times. A liter of saliva containing 5 grams of solid would be expected to contain $\frac{1}{250} \times 5000$ or 20 mg. of the purified substance as only 5 mg. was obtained the yield was approximately 25 per cent.

For studies on the chemical nature of the group substances Freudenberg, Eichel and Dirscherl⁶⁹ first used human urine as the source because of its easy availability. However this has the disadvantage that the amount of group-substance in urine

⁶¹ Schiff and Weiler *Biochem. Ztschr.* 235: 454 (1931).

⁶² *Klin. Woch.* 11: 1592 (1932); Schiff *Deutsch. med. Woch.* 59: 199 (1933).

⁶³ Landsteiner *Science* 75: 351 (1932); *Jour. Exp. Med.* 63: 185 (1936).

⁶⁴ Landsteiner and Chase *Jour. Exp. Med.* 63: 813 (1936).

⁶⁵ Cf. Freudenberg, Westphal and Graenewoud *Naturwiss.* 33: 522 (1936).

⁶⁶ Landsteiner and Harte *Jour. Exp. Med.* 71: 551 (1940). Cf. Landsteiner and Harte *Jour. Biol. Chem.* 140: 673 (1941); Goebel et al. *Jour. Biol. Chem.* 129: 455 (1939).

⁶⁷ *Jour. Immunol.* 41: 413 (1941).

⁶⁸ *Jour. Exp. Med.* 63: 185 (1936).

⁶⁹ *Naturwiss.* 20: 657 (1932).

is small the average inhibition titer for urine being 2^1 in contrast to a titer of 2^{11} for saliva (cf page 285). Accordingly even assuming a 100 per cent yield 250 liters of urine would provide only about 5 to 10 mg of the purified substance. Consequently it is not surprising that Freudenberg and his collaborators^{69a} subsequently obtained far more active preparations when they used commercial pepsin as the starting material than they had obtained from urine. On the other hand Friedenreich and Hartmann⁷⁰ report much higher titers for gastric juice (2^{12} to 2^{20}) than for saliva (average about 2^{11}). This suggests the use of gastric juice as a starting material and Witebsky and Klendshoj⁷¹ have obtained active preparations in this way.

Landsteiner and Harte⁷ studied the chemical nature of group substances from human saliva of secretors of groups A and B and O. The heated saliva was first evaporated to small volume at room temperature, freed of coagulable material and purified by repeated precipitation by alcohol from acid solution and re solution in water. From 500 c.c. of saliva were obtained 15 mg of material, soluble in water to form a viscous solution and possessing at least 40 times the activity of the total solids of saliva (a yield of about 25 per cent). A 1 per cent solution of the purified substance gave no precipitation with picric sulfosalicylic and trichloroacetic acids and basic lead sulfate the Millon reaction, Ehrlich test for tryptophane and lead test for sulfur were negative, the xanthoproteic and biuret tests faintly positive. The results of the chemical analysis⁷² were as follows:

Substance	Total N	Amino acid N	Hexoamine N	Hexoamine	Reducing sugar as glucose	Ash
A	5.65	2.48	1.81	23.3	45.5	0.16
B	5.33	2.35	1.71	21.7	48.5	0.90
O	5.74	2.91	1.68	21.5	46.5	1.91

Landsteiner and Harte pointed out that thus far no chemical differences have been found to explain the serological specificity since the analytical differences in the table are not significant. Studies on gastric juice from non secretors have been carried out by Witebsky and Klendshoj⁷ who extracted from these secretions polysaccharides of similar chemical structure to those present in the gastric juice of secretors.

^{69a} Freudenberg et al (footnote 65) Freudenberg and Westphal *Sitzungsber Heidelberger Akad Wissensch math naturwissensch Klasse* 1938 1 Abhandl

⁷⁰ *Ztschr f Immunstat* 92 141 (1938)

⁷¹ *Jour Exp Med* 72 663 (1940)

⁷² *Jour Biol Chem* 140 673 (1941)

⁷³ Cf Landsteiner and Harte *Jour Exp Med* 71 551 (1940)

⁷⁴ *Jour Exp Med* 73 625 (1941)

Witebsky and Klendshoj have also studied the reactions with anti O sera of group-substances extracted from gastric juice of secretors. These workers report that such preparations regardless of blood group all inhibit anti O sera about equally well while preparations from non secretors have no such action. The fact that also preparations from gastric juice of group AB secretors react with anti-O sera may have some bearing on the nature of the reactions of human erythrocytes of various groups with anti O sera. As Witebsky and Klendshoj, point out their results indicate that the reactions of the erythrocytes may not be due as believed by Thomsen to the presence of a separate O substance in bloods of group A and group B (cf page 204) based on heterozygosity (genotypes AO and BO) because under this assumption secretions from group AB individuals should not react with anti O sera.

Additional evidence with regard to the chemical nature of the group specific substances derives from their specific decomposition by certain bacteria. Thus Landsteiner and Chase⁵ showed that the activity of the A haptens is destroyed by Morgan's myxobacterium and *Saccharobacterium otale* microorganisms having the characteristic property of decomposing bacterial polysaccharides.¹⁶ According to Freudenberg and Eichel¹⁷ the specific substance is split by snail enzymes.

Reports by Jorpes¹⁸ must also be mentioned in which the protein nature of the substances from human urine which neutralize anti A iso agglutinins is suggested.

Summarizing the available evidence just presented one may assume that the structures responsible for the specificity of the blood group substances consist in part of carbohydrates, and that they occur in blood and organ cells in association with lipids to form alcohol soluble complexes. The lipids do not appear to be essential for the specificity, since fatty acids could not be recovered on hydrolysis of A substances present in horse saliva or pepsin.

Chemical information concerning the properties of M and N is lacking due to the fact that no method is yet known for extracting these substances from the red blood cells.¹⁹ Like the agglutinogens A and B the agglutinogens M and N are located in the stromata of the red blood cells and are resistant to heating and drying.

Artificial Antigens with Group Specific Action In recent experiments Morgan²⁰ has succeeded in preparing artificial antigens with A specificity. By fractional precipitation of 90 per cent phenol solutions of commercial pepsin or gastric mucin he first obtained non antigenic polysaccharides with pronounced A specificity. This he combined with the 'conjugated protein component' of the specific somatic antigen of *B dysenteriae* (Shiga²¹) to produce a powerful antigen which when

⁵ *Proc Soc Exp Biol and Med* 32 713 1208 (1935) Chase *Jour Bacteriol* 36 383 (1938)

¹⁶ Morgan and Thaysen *Nature* 132 604 (1933)

¹⁷ *Ann Chem Berlin* 518 97 (1935)

¹⁸ Jorpes and Norlin *Acta Path et Microbiol Scand* 11 91 (1934)

¹⁹ Cf Boyd *Jour Immunol* 27 485 (1934)

²⁰ *Chemistry and Industry* 60 722 (1941)

²¹ Morgan and Partridge *Biochem Jour* 34 169 (1940)

injected into selected rabbits (cf page 339) in doses of only 0.5 mg induced the formation of potent anti A agglutinins. This antigen, more over, had the advantage that it did not stimulate the formation of anti human species antibodies, so that group A cells were agglutinated by dilutions of the serum as high as and higher than 1:10,000, while B cells were not agglutinated by dilutions higher than the level for normal rabbit sera (average 1:12). Accordingly, Morgan considers

TABLE 65

SEROLOGIC RELATIONSHIP BETWEEN THE HUMAN AGGLUTINOGEN B AND AN AGGLUTINOGEN PRESENT IN RABBIT ERYTHROCYTES
(After Friedenreich and With)

Human Group A Serum	Dilution of Serum tested against Human B Blood							
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Serum no. 1, unabsorbed	+++	++	++	±	+	+	±	-
Absorbed } 1/16 vol B blood	-							
with } 1/16 vol rabbit blood	-							
Serum no. 2 unabsorbed	+++	++	++	++	+	+	+	-
Absorbed } 1/16 vol B blood	-							
with } 1/16 vol rabbit blood	++	++	+	±	tr	-		

such sera valuable for typing, especially for detecting weak A agglutinogens as in A_2B and A_1 bloods.

The antigen complex was formed either by precipitating the mixed components by alcohol from formamide solution⁸² or by mixing alkaline saline solutions (pH 8.9) of the two components and subsequently adjusting the pH to 4.5.

ON THE MOSAIC STRUCTURE OF THE AGGLUTINOGENS A AND B

Several investigations have revealed that cell antigens behave like a mosaic when they are tested with different immune sera. For example, it has been demonstrated that the agglutinogen A gives at least two distinct sorts of reactions, one of which is shared by the Forssman antigen (F_A) and the other peculiar to human A blood (cf page 31).

Similarly, human B agglutinogen exhibits several distinct serological properties (cf page 340) designated as B_1 , B_{11} , B_{111} , respectively⁸³ the first characteristic exclusively of human B blood and the others also found in the blood of certain lower animals. As Friedenreich and With⁸³ have shown the

⁸² Partridge and Morgan *Brit Jour Exp Path* 21: 180 (1940).

⁸³ The designation B_1 , B_{11} , B_{111} , etc. for the partial antigens of agglutinogen B has been adopted here in accordance with Boyd's suggestion [*Tabulae Biologicae* 17: 113 (1939)] instead of the designation B_1 , B_2 , B_3 , etc., proposed by Friedenreich, in order to avoid confusion with possible subdivisions in group B (cf page 218).

⁸⁴ *Ztschr f Immunitäts* 78: 152 (1933).

β agglutinins in many human group A sera can be completely absorbed by small quantities of rabbit blood as well as human B blood. However other group A sera cannot be exhausted even by large quantities of rabbit blood (cf table 65). This is explained by assuming that certain human A sera contain agglutinin β_{11} alone which can be absorbed by either rabbit or human B blood whereas other group A sera contain both agglutinins β_1 and β_{11} . Immune rabbit sera against human B blood contain agglutinin β_1 alone and testing fluids prepared from such immune sera react only with human B blood and not with animal blood. On the other hand chicken immune sera⁵⁴ against human B blood seem to contain only agglutinin β_1 .

Mention should also be made of a certain serological property common to blood cells containing agglutinogens A and B but absent from blood cells of group O. Thus Hooker and Anderson⁵⁵ were the first to notice that when certain anti B immune rabbit sera are absorbed with group O blood they still strongly agglutinate not only B blood but also A blood. Analogous results have been obtained with particular anti A immune sera.⁵⁶ Since a special agglutinin has been found to be present in these immune sera which is absorbable by bloods A, B and AB but not bloods of group O some workers have assumed the existence of a corresponding agglutinin C in bloods of groups A, B and AB and absent from group O blood. For reasons pointed out below a more likely interpretation is that the group-substances A and B have certain similarities in their chemical structure that are not shared with group-substance O.

The phenomena which have just been described have usually been accepted simply as evidence for the presence of a multiplicity of discrete agglutinogens in a single cell. However there is also the possibility that the reactions are due to different groupings in a single molecule and it has even been shown that the formation of multiple antibodies can be stimulated by substances in which the existence of distinct determinant groups can be excluded.⁵⁷ As Landsteiner remarks the Mendelian segregation of serological blood properties (not only A, B, M and N but also the many individual properties which have been discovered in animal blood) could not be understood without postulating discrete chemical substances. On the other hand failure of segregation for example of the so-called agglutinogens A and F_A of human group A blood, the antigens B_1 , B_{11} , B_{12} of human B blood and the so-called agglutinin C in bloods of groups A, B and AB favors the alternative explanation.⁵⁸

THE OCCURRENCE OF ISOAGGLUTININS OUTSIDE THE BLOOD SERUM

The general properties of the human isoagglutinins are not different from those of other agglutinins. Like other antibodies they are found in the globulin fraction of the serum. Accordingly, one would expect to find isoagglutinins only in those body fluids which are rich in protein. Thus,

⁵⁴ Cf Landsteiner and Witt *Jour Immunol* 11: 221 (1926).

⁵⁵ *Jour Immunol* 6: 419 (1921).

⁵⁶ Landsteiner and Witt *loc cit* *Hibino Ju enkwai Zasshi* (Japanese) 40: 4002 (1935).

⁵⁷ Cf Landsteiner [*Specificity of Serological Reactions* pp 60-72 (1936)] where these questions are discussed at length. Also see Hooker [*Jour Immunol* 33: 5 (1937)] and Morgan [*Jour Hyg* 37: 372 (1937)].

⁵⁸ Friedenreich [*Acta Path et Microbiol Scand* Suppl 37: 163 (1939)] remarks that he also is inclined to regard the different B characters as chemically related substances in the sense suggested by Landsteiner.

the isoagglutinins can be demonstrated in milk,⁸⁰ lymph, exudates transudates, the contents of cysts,⁸⁰ etc., although the titer, as a rule, is much lower than the titer of the isoagglutinins in the blood serum. There are only traces of isoagglutinins, if any, in the saliva, tears, or spermatic fluid.⁸¹ No isoagglutinins are demonstrable in urine, cerebrospinal fluid or amniotic fluid.⁸² They may be present in urine, however, under pathological conditions when the urine contains large amounts of protein.

TESTS FOR SPECIFIC SUBSTANCES OTHER THAN A AND B IN TISSUES AND SECRETIONS

In contrast to A and B, the properties M and N appear to be restricted to the erythrocytes in normal individuals. Thus Sasaki⁸³ and others⁸⁴ found that saliva does not possess the ability to inhibit the agglutinins for M or N, even in individuals secreting large quantities of A and B substances. In addition the properties M and N could not be detected in aqueous or alcoholic extracts⁸⁵ of normal tissues or in spermatozoa.⁸⁶ Zacho,⁸⁷ however, was able to demonstrate specific inhibition for M and N with saline extracts of malignant tumors.

Judging from the reactions of saliva⁸⁸ and semen⁸⁹ it would appear that the factor Rh, like M and N, is restricted to the blood cells—an observation of significance in relation to the etiology of erythroblastosis foetalis (cf. page 369). The author⁹⁰ has also found that saliva lacks the ability to inhibit the irregular isoagglutinin anti P. Boiled saliva can therefore be used for neutralizing the isoagglutinins α and β in human sera containing irregular isoagglutinins for P, Rh, M and possibly other factors, and in that way facilitates the use of such sera for diagnostic tests (cf. page 247).

⁸⁰ *Happ Jour Exp Med* 31: 313 (1920). *Heim Monatschr f Geburtsh u Gynak* 74: 52 (1926). *Schwarzman Ztschr f Geburtsh u Gynak* 92: 05 (1928) etc.

⁸¹ *Cavalieri Arch di Patol e Clin Med* 1: 5 (1922). *Weil and Isch Wall Compt rend Soc Biol* 88: 173 (1923) etc.

⁸² *Putkonen loc cit*. *Yosida loc cit*.

⁸³ *Ibid*.

⁸⁴ *Ztschr f Immunitats* 77: 101 (1932).

⁸⁵ *Boyd and Boyd Jour Immunol* 26: 489 (1934). *Wiener and Forer Proc Soc Exp Biol and Med* 47: 215 (1941) etc.

⁸⁶ *Boyd Jour Immunol* 27: 485 (1934).

⁸⁷ *Ztschr f Immunitats* 77: 520 (1932).

⁸⁸ *Wiener and Forer Proc Soc Exp Biol and Med* 47: 215 (1941).

⁸⁹ *Levine and Katzin Proc Soc Exp Biol and Med* 48: 126 (1941).

⁹⁰ Unpublished observations.

CHAPTER XVIII

ANTHROPOLOGICAL INVESTIGATIONS ON THE BLOOD GROUPS

BY MEANS of serological tests the proteins and cells of animals of any species can be differentiated as a rule, from those of animals belonging to other species. On the other hand attempts to produce sera which would serve to differentiate bloods of different races particularly in the human species have been unsuccessful.

Agglutinogens of human blood so far discovered are not restricted to any race and consequently the presence or absence of any of the agglutinogens in a given blood specimen cannot be used as evidence that the blood came from an individual of a given race. However the study of the agglutinogens in the bloods of large groups of individuals has shown that their *frequency distribution* varies in different races.

Racial Distribution of the Blood Groups The first studies on the racial distribution of the blood groups were made by L. and H. Hirszfeld¹ during World War I. These workers had the opportunity, as army physicians, to test blood samples of soldiers and civilians of different races concentrated in the Balkans. They examined 500 to 1000 individuals of each nationality and found that the frequencies of the four groups varied in the different peoples and were related to a certain extent to the geographic locations of their countries. The Hirszfelds reported these results in graphic form as shown in figure 59. A detailed analysis of their data in connection with the theories of heredity of the blood groups has been given in tables 21 and 22.

The Hirszfelds pointed out that the frequency of the agglutinin A decreases from west to east while the frequency of agglutinin B increases. They also found that the 16 peoples studied could roughly be subdivided into three groups on the basis of the relative frequency of the factors A and B. The ratio of A to B was called the *biochemical index* and was calculated by dividing the sum of the frequencies of groups A and AB by the corresponding sum for groups B and AB.

$$i.e. I = \frac{A+AB}{B+AB}$$

On this basis three types were distinguished: a European type with an index of 2.5 or more; an intermediate type with an index of 1.3 to 1.8; and an Asia-African type with an index of 1.0 or less. In subsequent studies transitions between these types were found so that no sharp divisions really exist.

Various authors have suggested other indices to express the serological composition of races. Since indices of this nature which attempt to express the distribution

¹ *Lancet* 2: 675 (1919)

of the blood groups in terms of a single number give an incomplete picture they should be discarded. Thus the biochemical race index of the Hirszfelds would not

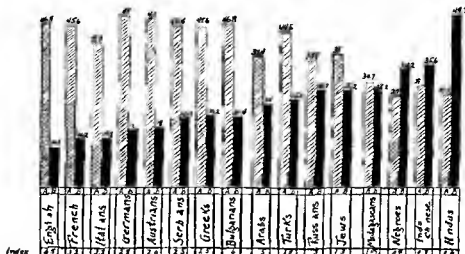


FIG 59 DISTRIBUTION OF THE AGGLUTINOGENS A AND B
(After H and L Hirszfeld)

differentiate two races having the same relative frequencies of the factors A and B although the frequencies of the four groups differed widely in the two races as in the following example

TABLE 66

Race	Per Cent of Groups				Index
	O	A	B	AB	
Caucasians	31.9	40.0	23.1	5.0	1.6
Indians, Peru	75.1	14.1	7.2	3.6	1.6

The reason for this is that the distribution of the groups in a population is determined only if two independent figures are given. Thus the group distribution may be calculated if one knows the frequencies of two of the three genes (cf. page 186) since they are connected by the relation $p + q + r = 1$.

During the past 15 to 20 years studies have been made on the distribution of the blood groups in practically every race in the world. Hundreds of investigations of this sort have been made, a selection of which is listed in table 67. In cases where several studies had been made on the same population those results were selected which include the largest number of individuals. For example only the more representative of the many studies made in Germany are given. Because of the large number of investigators who took part in this work, the technic

TABLE 67
RACIAL DISTRIBUTION OF THE BLOOD GROUPS*

Nationality	Investigators	of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Abyssinian	Winkel	400	42.8	26.5	25.2	5.5	17.5	16.8	65.4
Anu Hidaka	Grove and Nanomya	509	17.0	31.8	32.4	18.4	29.7	30.1	41.2
Anu Hidaka	Arai	563	16.9	31.8	32.9	18.5	29.1	29.9	41.0
Anu Karafuto	Ishii	205	37.1	24.4	32.1	5.8	16.5	21.6	60.9
Anu Karafuto	Arai	336	33.3	28.9	26.2	11.6	22.1	20.2	57.7
Anu Piratori	Grove	240	11.6	29.3	34.1	25.0	31.9	32.4	34.1
Anu Tokachi	Arai	754	27.6	47.5	18.7	6.1	33.1	15.5	52.5
Albanians (Sicily)	Nicoletti	500	37.8	43.0	13.2	6.0	27.9	10.2	61.4
American Indians									
Araucanos (Chile)	Onetto-Castillo	382	75.6	17.2	6.2	0.6	9.5	3.7	86.9
Argentina	Mazza and Frank	94	82.9	12.8	4.3	0.0	6.7	2.2	91.0
Blackfeet and Blood tribes (pure)	Matson and Schrader Matson Levine Schrader	394	22.8	76.7	0.0	1.0	54.4	0.5	45.5
British Columbia	Gates and Darby	300	36.7	12.7	0.6	0.0	6.6	0.3	93.1
Caraja	Golden	61	39.0	5.0	51.0	5.0	4.0	34.0	62.0
Flatheads (Montana)	Matson and Schrader	258	51.5	42.2	4.7	1.6	25.0	3.3	71.8
Mexican	Moss and Kennedy	318	39.2	27.1	11.8	1.9	16.0	7.1	76.8
Navajo	Allen and Korberr	622	69.1	30.6	0.2	0.0	16.6	0.1	83.3
Navajo	Nigg	517	70.8	28.6	0.3	0.1	15.5	0.3	84.2
Papago	Brazzale Green and Kantor	600	93.8	6.2	0.0	0.0	3.1	0.0	96.9
Peru (pure)	Laretta	200	100.0	0.0	0.0	0.0	0.0	0.0	100.0
Peru (half breeds)	Laretta	1 372	75.1	14.1	7.2	3.6	9.3	5.6	86.7
Pueblo (Keresen)	Allen and Schaeffer	353	88.0	11.7	0.0	0.3	6.1	0.1	93.8
Pueblo (N M)	Allen and Schaeffer	307	84.5	13.3	1.6	0.6	7.0	1.1	92.0
Sioux	Matson	260	68.1	29.0	2.7	0.2	15.9	1.5	82.5
U S	Coca and Debert	862	77.7	20.2	2.1	0.0	10.7	1.1	88.0
U S (pure)	Snyder	453	91.3	7.7	1.3	0.0	3.8	0.5	98.3
Ute	Matson and Schrader	136	92.4	2.5	0.0	0.0	1.3	0.0	98.7
Yucatan (pure)	Goodner	223	97.7	1.3	0.5	0.5	1.0	0.5	98.8
Yucatan	Moss and Kennedy	738	76.6	16.7	5.4	1.4	10.3	3.7	86.3
Yamasee	L and H Hirschfeld	500	47.0	22.4	28.4	7.2	16.1	19.8	64.8
Arabs Mohammedan (Beirut)	Parr	793	33.8	36.8	18.4	10.8	27.8	16.0	58.2
Arabs, Christian (Beirut)	Parr	905	32.1	44.0	11.2	9.5	31.8	11.0	39.2
Arabs (Syria)	Altounyan	933	35.7	37.0	20.8	6.5	24.8	14.8	59.8
Arabs Cyrenaica	Tedeschi Medulla Mazzola Todda	2 917	44.0	53.0	17.7	4.1	21.5	12.2	66.4
Arabs (Baghdad)	Boyd and Boyd	500	34.1	30.8	28.9	6.2	22.1	20.9	58.6
Argentimans (Buenos Aires)	Espejo Sall	431	49.8	39.2	7.8	3.2	23.9	3.4	70.5
Argentineans (Buenos Aires)	Mazza and Frank	651	49.0	39.0	9.5	2.5	23.5	6.2	70.0
Armenians (Aleppo)	Altounyan	653	27.0	53.0	14.0	6.0	36.8	10.6	51.0
Armenians Tiflis	Semenskaya	308	33.8	49.0	10.1	7.1	33.0	9.0	58.1
Armenians Beirut	Parr	1,536	28.3	46.7	12.6	12.4	36.1	13.4	53.2
Armenians (Ideschwan)	Mirsoyan	669	30.8	53.8	10.3	5.1	36.4	8.6	55.5
Assyrians	Parr	147	32.2	43.6	18.6	9.4	31.6	12.9	56.8
Australian aborigines	Cleland	398	35.9	66.1	0.0	0.0	41.7	0.0	58.3
Australian aborigines (full-blooded)	Birdsell and Boyd	805	53.1	44.7	2.1	0.0	26.0	1.4	72.9
Australians (Queensland)	Lee	377	60.3	31.7	6.4	1.6	18.3	4.0	77.8

* For bibliography see Boyd's *Blood Groups* [Tables Biologicas 17 Part 2 (1939)] also references at end of chapter

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Australian whites (Sidney)	Shpton	200	41.8	43.6	9.1	2.7	26.7	6.1	66.8
Austrians	Hoche and Moritsch	1,000	33.1	39.0	20.1	6.9	27.2	14.6	57.4
Bantu (S. Africa)	Piiper	808	53.2	25.3	19.2	2.3	15.0	11.4	72.9
Bantu	Edison Drew	5,000	54.5	23.6	19.7	2.2	13.8	11.6	73.8
Bashkirs	Vagner	155	20.8	36.2	25.5	8.5	25.6	19.0	54.7
Basques (San Sebastian)	Boyd and Inzar	229	56.8	39.7	1.3	2.2	22.8	1.8	75.4
Bedouin Syrian	Boyd and Boyd	304	39.8	23.4	30.2	6.6	16.4	20.5	63.2
Bedouin (near Baghdad)	Kayser, Boyd and Boyd	338	40.8	26.8	25.8	6.8	18.1	17.7	63.9
Belgians	Straquet	1,072	47.9	41.8	7.1	3.2	25.8	5.3	69.2
Belgians (Liege)	Mouveau	3,500	46.7	41.9	8.3	3.1	25.9	5.9	68.3
Belgian Congo									
Alar	Jadin	512	40.6	38.5	17.0	3.9	25.2	12.2	63.8
Babara	Jadin	275	53.1	26.0	18.7	2.2	16.1	11.8	72.9
Balese	Jadin	507	48.5	30.8	18.4	4.3	19.4	10.9	69.7
Pygmies (Lé speech)	Jadin	1,032	30.8	30.3	29.1	10.0	22.7	21.9	55.4
Pygmies	Julien	1,013	27.1	35.9	28.1	8.9	27.3	22.2	52.1
Berbers (Morocco)	Benot and Kossowatz	4,947	39.2	38.9	16.7	5.2	25.2	13.6	62.6
Berbers (Tunisia)	Caillon and Didier	500	48.4	32.4	15.8	5.4	20.8	10.6	68.2
Brahmans (Bonokromo Java)	Buning	82	37.8	22.4	32.0	7.8	16.0	22.0	61.4
Brazilians (Sao Paulo)	Bier and Machado	4,000	47.1	40.4	9.1	2.6	24.8	8.4	69.1
Brani (whites) Para	Aben Athar	274	50.0	29.5	17.8	2.5	17.7	10.9	70.1
Brazil, So Paulo	Lefèvre	287	46.0	36.0	15.0	3.0	22.0	9.5	67.8
Buginese Celebes	Lehmann	217	34.8	30.4	27.6	7.4	21.1	19.4	58.8
Bulgarians	L. and H. H. Isfeld	500	30.0	40.6	14.2	8.2	27.1	10.8	62.4
Bulgarians Sofia	Senow and Zentschew	1,000	30.6	41.8	12.5	8.1	31.4	13.2	55.3
Bulgarians Sofia	Ganev	8,060	32.1	44.4	15.4	8.1	30.8	12.3	56.7
Burnats (eastern)	Zhunkin	1,260	28.4	22.5	40.0	11.3	18.4	30.1	51.4
Burnats (western)	Melikh	1,602	33.3	20.9	37.5	8.3	15.8	26.4	57.8
Bushman	Piiper	815	56.1	29.6	5	8.8	20.2	7.4	74.9
Carehans	Zolotarew	691	39.2	31.0	21.0	5.0	20.4	16.2	62
Catalans	Grafols and Ross	1,000	41.7	48.0	8.2	2.1	28.4	5.4	61.4
Caucasus eastern									
Kakhetians (Tiflis)	Semenskaya et al.	1,274	36.8	42.3	15.0	5.9	28.3	11.3	60
Kartabians (Tiflis)	Semenskaya et al.	8,268	40.6	42.2	12.1	5.1	21.3	8.9	63.8
Caucasus western									
Adzharians (Tiflis)	Semenskaya et al.	107	59.1	34.4	6.1	0.4	19.8	3.8	16.0
Gurians (Tiflis)	Semenskaya et al.	1,858	58.8	30.0	9.6	1.8	17.6	6.0	18.6
Imertians (Tiflis)	Semenskaya et al.	2,916	52.0	34.8	10.7	2.5	21.1	7.1	72.1
Mingrelians (Tiflis)	Semenskaya et al.	1,362	58.6	30.7	8.0	2.7	17.9	5.1	76.6
Chileans	Meza et al.	242	52.9	12.8	32.0	1.7	7.6	18.9	72.1
Chinese Hunan	Li-Chi Pao	1,500	31.3	38.1	20.7	9.9	2.9	16.7	55.9
Chinese Peking	Lau Wang	1,000	30.0	25.0	35.0	10.0	20.0	25.8	54.8
Chinese Sumatra	Dais and Verhoef	592	40.2	25.0	27.6	7.2	17.7	19.3	63.4
Chinese, Canton	Dormans	1,000	45.5	22.8	25.0	8.1	16.0	17.4	6.4
Chinese riverside populations	Yang Fung Mun	5,418	38.4	30.3	23.4	7.8	21.1	16.9	62.0
Chinese Shansi along lines	Curran Rosenow and Feng	1,000	32.5	22.2	34.0	11.3	18.4	26.0	57.0
Chinese (Formosa)	Yang Fung Mun	2,127	34.2	30.8	24.7	7.3	22.0	20.1	58.1
Chuvaks	Wachniewsky	951	32.8	26.3	34.8	6.3	18.0	23.2	57.2
Czechs (Bratislava)	Trohan	1,164	32.8	51.1	10.0	5.5	34.5	8.0	37.3
Czechs (Brno)	Suk	3,010	28.8	44.0	18.2	9.0	31.7	11.9	51.1
Dahur (Manchuria)	Kundusa and Makino	595	27.1	29.9	32.3	10.7	21.9	31.5	52.1
Danes	Hansen	1,000	37.0	46.4	11.8	4.8	30.1	8.7	60.8

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Danes Copenhagen	Thomsen	1,151	41.1	43.1	8.3	4.5	27.6	6.6	66.4
Danes Copenhagen	Sand Munk, and Knudtzon	1,759	42.8	42.4	11.3	3.5	26.3	7.8	65.5
Danes	All authors combined (after Streng)	16,037	42.0	43.6	10.4	3.9	27.6	7.5	64.8
Douret (Tunisia)	Caillon and Discher	343	58.3	32.6	7.8	1.1	18.7	4.7	76.4
Druze (Beyrouth, Syria)	Farr	229	33.2	56.2	18.3	12.2	25.7	14.1	57.6
Dutch	v. Herwerden	6,679	46.8	41.7	8.6	3.0	25.3	6.0	68.5
Dutch, S. Africa	Paper	596	45.8	39.6	11.9	2.7	24.0	7.6	67.8
Dutch	All authors combined (after Streng)	14,483	46.3	42.1	8.5	3.1	26.0	6.0	68.0
Egyptians	Shousha	417	24.2	32.6	29.2	13.9	27.0	24.5	49.2
Egypt									
Alexandria	Partheniades	460	30.4	48.7	14.1	6.7	33.8	11.6	55.3
Copts (Assiut)	Boyd and Boyd	419	24.6	31.4	31.0	10.0	27.2	23.0	49.6
Copts	Moharram	1,476	31.2	33.9	24.7	10.3	24.7	18.8	55.9
Moslems (Cairo)	Matta	754	26.6	35.8	27.1	10.5	27.4	21.7	51.6
Moslems (Cairo)	Boyd and Boyd	502	27.3	38.4	25.3	8.8	28.8	20.3	52.3
England (E. Counties)	Peatrose and Peatrose	1,000	43.2	47.7	6.4	2.7	29.5	4.8	65.8
England (North)	Taylor and Prior	422	47.9	42.4	8.3	1.4	25.0	5.0	69.2
English, Shenley	Thomas and Hewitt	900	42.1	43.8	10.1	4.0	27.8	7.4	64.9
English, Slough	Vaughan	4,032	49.8	39.3	8.3	2.6	23.8	5.6	70.6
English, London	Kirwan-Taylor	500	40.4	46.8	9.6	3.2	29.3	6.7	63.6
English, Liverpool	Jones and Glynn	1,600	46.0	30.0	17.0	7.0	20.5	12.8	67.7
English, Australia	Tebbut	1,176	52.6	36.8	7.4	3.0	22.5	5.5	72.6
Eskimos, pure									
(East Greenland)	Fabricius-Hansen	569	23.9	56.2	11.2	8.7	40.6	10.6	48.9
Eskimos (Baffin Land)	Heimbreyer and Pauls	166	55.4	43.6	0.6	0.6	25.2	0.6	74.4
Eskimos (West Green- land)	Bay Schmidt	201	41.1	53.8	3.5	1.4	33.2	2.5	61.1
Eskimos (Jacobshaven Greenland)	Freuchen	340	43.5	47.1	7.3	2.1	28.7	4.8	65.9
Eskimos (Cape York Thule, etc.)	Heimbreyer and Pauls	121	80.7	12.9	2.4	4.0	8.9	3.2	89.8
Eskimos (S. W. Green- land)	Fabricius-Hansen	1,063	36.3	54.6	5.3	3.7	35.1	4.3	60.3
Eskimos (Cape Farewell Greenland)	Bay-Smith	607	51.2	38.5	4.8	2.0	22.5	3.4	74.2
Estonia (Central and South)	Rooks	1,841	32.3	36.6	22.4	8.7	26.1	17.0	57.0
Estonia (North)	Raukas-Poska	849	34.2	33.3	26.2	6.3	23.7	19.2	58.5
Filipinos, Igorot	Grove	214	51.6	21.4	21.4	5.6	15.2	15.2	71.0
Filipinos, Samal Moros	Grove	501	25.9	18.1	14.9	11.1	16.0	33.8	49.3
Filipinos, Negritos	Grove	297	18.4	33.3	14.1	4.0	20.9	9.6	69.6
Filipinos, Bogobo	Grove	302	33.7	16.9	26.5	2.9	10.5	16.1	73.2
Filipinos, Sulu	Grove	412	41.6	23.0	30.3	4.9	15.2	19.6	64.5
Filipinos	Cabrera and Wade	204	64.7	14.7	19.6	1.0	8.2	10.9	30.4
Finnish speaking	All authors combined (after Streng)	23,200	34.1	41.0	13.0	6.9	27.9	13.4	58.7
Uusimaa, Turku-Parl Häme Laasa Ahve nänmaa									
Väpärä Mikkeli Kus- po Oulu Lappa		11,330	29.7	44.2	18.1	8.0	31.1	14.1	54.8
Finnish Swedish-speaking		8,254	35.2	43.3	16.2	5.3	28.6	11.5	59.9
Formosan, Pawan	Kondo	335	42.4	18.2	32.2	7.2	12.8	21.3	65.1

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Formosans Ami	Kirihara and Haku	231	37.2	26.0	24.2	12.6	21.7	20.5	60.9
Formosans Ami	Kirihara and Haku	236	22.1	41.1	13.6	43.2	52.4	17.6	52.0
Formosans (from Fukien)	I uruchi	404	47.0	23.0	23.5	6.7	15.2	15.4	68.5
French	L. and H. Harszfeld	500	43.2	42.6	11.2	3.0	26.2	7.4	63.7
French	Kossovitch	962	42.1	42.3	11.1	4.5	27.0	8.1	64.9
French	Parr	1,197	40.9	41.4	10.0	7.7	28.7	9.3	64.0
French (Alsace)	Grooten and Kossovitch	424	37.2	48.4	9.4	4.5	31.5	7.6	61.0
French (Lille)	Farjot	600	43.8	45.8	8.0	2.3	28.4	5.8	66.2
French (North)	Balgares and Christaen	3,000	43.9	42.2	10.5	3.4	26.5	7.5	66.3
Georgians (Tiflis)	Boyd and Boyd	500	49.0	35.8	13.2	4.0	21.0	8.9	70.0
Germans Berlin	Schiff	39,174	36.5	42.5	14.5	6.5	28.5	11.0	60.4
Germans Cologne	Wichmann and Paal	1,100	40.2	46.5	11.0	2.5	27.2	7.0	64.8
Germans Danzig	Wagner and Kruse	400	29.5	44.7	17.0	8.5	32.0	14.3	54.5
Germans Duisberg	Runkel	5,036	40.7	41.6	10.0	4.7	28.6	7.4	63.8
Germans Erlangen	Kruse	1,600	40.5	45.9	10.6	5.0	28.5	7.0	63.7
Germans Eisen	Leweringhaus	2,000	38.1	45.7	11.7	4.3	29.5	8.5	61.7
Germans Frankfurt a M.	Fischer	3,000	41.5	43.8	10.8	4.0	27.8	7.7	64.7
Germans Leipzig	Cited after Arnold	8,000	37.1	42.9	15.5	4.5	27.7	10.7	61.2
Germans Munich	Kruse	1,300	42.5	43.0	9.6	4.9	26.9	7.6	65.3
Germans Saxony	Christiansen	33,295	38.9	42.1	13.7	5.4	27.3	10.0	62.4
Germans Schleswig Holstein	Gundel	19,480	59.4	45.4	12.4	4.7	28.2	9.0	62.8
Germans, Württemberg	Mayer	1,300	45.0	42.4	9.9	4.7	27.3	7.6	63.7
Germans (Germany)	All authors combined (after Streng)	172,701	39.1	45.5	12.5	4.9	28.2	9.1	62.5
Germans Innsbruck	Holzer	20,068	41.8	43.7	10.6	3.9	27.8	7.7	64.6
Germans Kul	Gundel	10,511	38.4	45.2	13.4	5.0	28.4	10.0	62.0
Germans Austria, Vienna	Corvin	8,934	34.4	45.2	13.0	5.4	30.1	11.2	58.7
Germans (Austria)	All authors combined (after Streng)	21,274	35.8	45.4	14.9	6.1	28.9	11.1	59.7
Germans (Switzerland)	All authors combined (after Streng)	7,715	40.6	48.6	9.6	5.2	29.1	6.6	63.7
Germans Silesia	Grötschel	1,600	35.1	39.7	18.1	7.0	27.0	16.2	59.3
Gilaks	Kisha	62	50.0	27.4	14.5	8.0	19.7	12.1	70.7
Greeks	L. and H. Harszfeld	500	38.2	41.8	16.2	4.0	26.2	10.7	61.8
Greeks	Kumaris	4,398	40.0	41.6	13.7	4.7	26.7	9.6	63.3
Greeks Athens	Diamantopoulos	1,200	42.0	37.6	14.2	3.7	25.4	10.2	64.8
Grusenes	Semenskaya	2,178	49.2	37.4	12.0	4.4	23.7	8.6	68.0
Gypsies (Hungary)	Gärtner	925	28.5	26.6	55.3	9.6	20.8	26.5	53.4
Gypsies (Hungary)	Vérzar and Weiszecsky	383	31.2	21.1	38.9	5.8	14.5	25.6	58.4
Gypsies (Hungary)	Hesch	102	26.5	27.4	37.5	8.8	20.1	26.6	51.5
Gypsies (Vovodina)	Schmidt	529	35.9	29.3	26.1	8.7	21.3	19.3	59.9
Haitians Santo Domingo	Moss and Kennedy	111	58.7	24.7	13.8	2.8	14.9	8.6	76.6
Hindus "North"	L. and H. Harszfeld	1,000	31.3	19.0	41.2	8.5	14.9	29.1	56.0
"Hindus" South	Bau and Verboef	348	37.9	23.0	31.6	7.5	16.6	22.0	61.5
"Hindus"	Malons and Lahiri	2,357	30.2	24.5	37.2	8.1	17.9	26.1	54.9
Hottentots, Pretoria	Piper	500	34.8	30.6	29.2	5.3	20.0	19.0	59.0
Hungarians	Kus	4,212	35.2	40.5	17.9	6.3	27.4	13.2	59.4
Hungarians Debrecen	Vérzar and Weiszecsky	1,500	31.0	38.0	18.8	12.2	29.4	17.0	55.6
Hungarians, Budapest	Weitzner	1,000	35.7	43.3	15.7	5.3	28.3	11.5	59.8
Hungarians (Budapest)	Lenst and Latja	624	36.1	41.8	15.9	6.2	28.0	11.3	60.4
Hungarians (Bratislava)	Trokan	1,495	29.1	49.6	15.9	7.8	54.7	11.6	54.0
Hungarians (Dudar)	Mehegy	473	28.0	32.9	12.6	8.5	57.8	11.1	51.0
Hungarians (Muresch)	Ramnesstru	1,318	27.6	42.5	19.5	10.6	31.2	15.9	52.5
Hungarians (Ofenpest)	Kus	4,242	35.3	40.5	17.9	6.3	27.6	13.5	59.4

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Icelanders	Jonsson	800	55.7	32.1	9.6	2.6	19.3	6.3	74.6
India†									
Rajput (Indo-Aryan)	Malone and Lahiri	188	28.8	28.0	33.0	10.2	21.7	25.0	53.7
Mahishya (Budge Budge, Bengal)	Macfarlane	160	32.5	20.0	39.4	8.1	13.4	2.8	57.1
"Dravidians" (Munda Santal and Araon)	Malone and Lahiri	589	24.3	27.5	36.8	11.4	21.8	28.0	49.3
Bhil (Central provinces)	Macfarlane	140	18.6	23.6	41.4	16.4	21.8	14.4	43.1
Paniyans	Aiyappan	250	20.0	62.4	7.6	10.0	46.1	7.8	41.7
Kanikkar	Kartunakaran	211	51.2	18.5	29.9	0.5	12.0	18.5	71.5
Angami Nagas (Assam)	Mitra	165	46.1	38.8	11.5	3.6	24.2	8.1	68.0
Flamingmen (Assam)	Mitra	2,000	33.7	24.6	52.5	9.2	18.3	22.3	58.2
Malayali (lower Cochun)	Macfarlane	260	43.1	29.3	16.1	6.5	18.6	10.8	69.6
Toda	Pandit	200	29.5	19.5	38.0	15.7	15.7	27.8	54.5
Indochina									
Lhas	Farnaud	690	26.7	28.6	32.3	12.5	22.7	25.1	51.7
Phnonga	Farnaud	599	26.3	27.1	33.1	13.5	21.8	25.8	51.3
Siangs		508	23.2	29.5	35.6	11.6	24.4	28.5	48.2
Indochina (North)	Marneffe and Bezacier								
Annamites		1,261	44.2	19.8	23.9	7.1	14.3	20.0	66.3
Muong		506	37.4	24.7	31.0	6.9	17.3	21.2	61.2
Tho		572	45.3	20.3	29.9	4.5	13.3	19.0	67.3
Indonesia									
Galela	Björner	406	57.0	24.0	15.0	4.0	14.5	9.4	75.6
Tobelo	Björner	450	51.0	27.0	18.7	3.5	16.9	12.1	71.4
Iranians (in Samarkand)	Libman	500	30.6	31.8	31.6	6.0	23.7	23.5	55.3
Irish (Dublin)	Sachs	2,135	53.6	32.4	11.5	2.5	19.3	7.3	73.2
Irish (North)	Taylor	354	58.5	27.7	12.1	1.7	16.0	7.2	76.3
Irish (Dublin)	Boyd and Boyd	399	53.2	31.1	12.0	1.7	18.6	7.6	74.4
Italians (all)	After Lattes	17,157	41.2	39.4	14.0	5.4	25.7	10.5	64.2
Italians Northern and Central	(Twenty six authors)	11,227	42.0	43.4	10.6	4.0	27.5	7.7	64.8
Italians Sicily		1,109	43.2	34.9	17.1	4.9	22.6	11.9	65.7
Italians (Sassari Sardinia)	Manaj and Simola	968	49.8	26.4	18.9	4.9	16.7	12.3	50.5
Italians (W. Sardinia)	Benassi and Azzena	500	56.6	33.4	8.8	1.2	19.7	5.7	75.3
Japanese (in Japan)	After Furubata								
District Ou	All authors combined (1916-1933)	24,672	31.1	36.7	22.7	9.5	26.6	17.6	55.7
District Kwanto		49,749	30.5	37.7	22.1	9.7	27.4	17.4	55.2
District Kokuniku		46,600	31.4	36.6	22.4	9.6	26.6	17.4	56.0
District Tokai		33,834	31.2	38.4	21.8	8.6	27.4	16.8	55.8
District Kinkai		34,369	30.5	38.3	21.7	9.5	27.7	17.0	55.2
District Chugoku		24,152	28.4	40.6	21.6	9.4	29.5	17.2	53.3
District Shikoku		18,551	29.0	40.8	21.3	8.9	29.4	16.8	53.8
District Kyushu		64,608	30.4	38.7	21.5	9.3	28.0	16.9	55.2
District Hokkaido		6,360	31.7	36.0	22.5	9.9	26.2	17.5	56.3
Japanese (outside Japan)									
Formosa		3,551	35.0	31.7	22.4	7.9	24.3	16.6	59.2
Dauren		3,906	26.9	40.8	21.6	10.6	30.4	17.7	51.9

† A detailed study of this heterogeneous group of peoples has been made by Macfarlane [*Jour. Gen.* 36: 225 (1938), *Amer. Jour. Phys. Anthropol.* 25: 397 (1941)].

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Mukden		296	29.7	3.4	22.1	10.8	27.5	18.1	54.5
Korea		2 537	29.0	37.7	25.0	8.5	21.0	22.1	53.9
Japanese (totals)		301 959	30.5	38.2	21.9	9.4	27.6	17.1	55.2
Javanese	East and Verhoef	1 346	39.9	25.7	29.0	5.4	17.1	19.1	63.0
Javanese									
Modjowarno	Buning	4 128	37.2	24.4	32.0	6.4	17.5	22.2	61.0
Getaian	Buning	832	38.7	23.2	31.0	7.3	16.4	21.3	62.3
Dampet	Buning	233	83.6	22.8	47.2	8.4	14.2	10.9	73.2
Javanese (Sourabaya)	Buning	8.3	35.2	20.8	35.7	8.3	15.5	24.8	59.4
Jews Dutch	v. Herwerden	95	42.8	39.4	13.4	4.5	25.2	9.4	65.3
Jews Polish	Halber and Mydlarski	818	33.1	41.8	17.4	8.0	28.0	13.8	58.0
Jews Roumanian	Jonescu	1 435	38.2	39.0	17.5	6.3	25.3	12.2	61.8
Jews Macedonian	L. and H. Hirsfeld	500	38.8	33.0	23.2	5.0	21.3	15.3	62.3
Jews Russian Odessa	Barinstein	1 475	36.6	41.7	15.8	8.1	27.8	11.3	60.5
Jews Ashkanazim (Tel Aviv Palestine)	Younovitch	1 500	34.0	41.1	18.1	6.8	28.3	13.9	38.3
Jews Yemen (ta)	Younovitch	1 000	56.0	26.1	16.1	1.8	15.7	10.1	74.8
Kalmucka	El'remoff	214	25.7	22.9	40.6	10.8	17.7	30.2	50.8
Karghis	Koopman	738	31.4	29.1	30.4	8.1	19.5	20.3	48.7
Karghis	Pussarow	914	36.8	24.0	31.4	9	17.4	22.0	60.1
Koreans Hsiao	Kurahara	948	28.3	32.7	32.2	8.8	23.5	23.2	51.4
Koreans	All authors combined (after Furuhashi)	9 434	27.7	31.3	30.7	10.0	23.9	23.4	32.8
Lapps Sweden	Schött	404	28.9	62.6	4.4	3.9	42.0	4.5	53.9
Letts	Weidemann	1 160	51.6	58.2	24.2	8.9	14.8	17.0	5.1
Lithuanians	Woblfell	200	39.0	40.0	18.0	3.0	21.5	11.1	62.3
Lithuanians	Jurgelunas and Ravensberg	1 582	36.8	40.0	19.5	3.7	23.0	12.4	80
Lithuanians (Kaunas)	Zilinskas and Masalakis	2 452	40.4	34.1	20.0	5.8	22.8	14.3	63.4
Madagascan	L. and H. Hirsfeld	500	45.3	26.3	23.7	4.3	18.8	15.3	8.5
Magyars (Szegedin)	Rosatoory	840	31.5	43.6	16.8	8.3	30.5	13.7	58.1
Malays (Celebes)	Lehmann	412	31.8	30.2	29.2	8.8	21.8	21.3	58.4
Manchu Mukden	Kurahara and Hake	236	30.9	25.9	33.9	9.3	19.4	24.6	55.6
Manchu	Fukamachi	490	26.7	26.6	38.2	8.5	19.8	2.0	51.8
Mari	Petroff	190	22	22.7	3.3	17.3	23.1	32.9	47.8
Melanesians, New Guinea	Heydon and Murphy	753	33.7	26.8	16.3	3.2	18.4	10.3	73.3
Melanesia									
Ambon	B. Jömer	4 474	53.9	20.9	20.9	2.3	12.8	12.8	71
Aloana (Ceram)	B. Jömer	65	34.0	42.0	17.1	6.9	28.8	13.2	58.3
Lomna (Pantar Is.)	Brouwer	699	51.9	30.6	11.2	3.3	18.7	9.3	72.1
"Papuan" (N. Bial)	Bos	48	82.7	18.5	18.7	2.1	10.9	9.9	9.2
"Papuan" (N. Soepora)	Bos	613	63.7	45.4	18.5	2.4	9.2	10.8	79.8
Melanesians New Guinea	Heydon and Murphy	753	53.7	26.8	16.3	3.2	18.4	10.3	73.3
Micronesians	After Furuhashi	3 604	46.8	33.8	16.7	4.7	21.7	11.4	66.9
Marshall Is.	Takasaka	843	52.2	28.0	18.9	2.9	1.3	10.8	72.3
Mohammedans Tunis	Caillon and Discher	500	46.8	37.4	15.8	5.4	21.2	11.2	68.1
Moors (Algeria and Tunis)	L. and H. Hirsfeld	500	43.8	32.4	19.0	5.0	20.9	12.9	66.0
Moors (Morocco)	Bernot and Komovitch	527	37.2	36.8	19.9	6.4	24.4	11.1	61.0
Negroes also see Bantu									
Negroes, Bantu	Fine	230	52.0	27.2	49.2	1.8	15.6	11.1	2.1
Negroes Bush (Dutch Guinea)	Kahn	336	83.0	0.0	17.0	0.0	0.0	8.9	91.0
Negroes, Congo (Kafanga)	Bruyoghe and Walravens	500	45.8	22.2	21.2	8.0	16.5	17.7	6.5

TABLE 67 (Continued)

Nationality	Investigators	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Negroes Middle Congo	L. edt and Poparski	400	41.0	27.0	26.0	6.0	18.2	17.6	64.0
Negroes West Africa	Lewis and Henderson	325	52.3	21.5	23.0	3.2	13.3	14.3	72.4
Negroes North America	Snyder	500	47.0	28.0	20.0	5.0	18.2	13.4	68.5
Negroes Jamaica	Snyder	144	46.5	29.1	22.2	2.1	17.2	13.1	68.2
Negroes Senegal	L. and H. Hirschfeld	500	42.2	22.4	29.2	5.0	14.9	18.9	65.7
Negroes (N. Y. City U.S.A.)	Landsteiner and Levine	750	44.2	30.3	21.8	3.7	19.8	14.8	66.5
Negroids (Tunis)	Caillon and Diacher	500	46.4	32.4	15.8	5.4	21.2	11.4	68.1
Norwegians	Jervell	436	35.6	49.8	10.3	4.3	32.3	7.6	59.7
Norwegians	Hartmann and Bjerke-Jund	10 000	39.2	48.7	8.5	3.5	31.2	6.5	63.6
Norwegians (N. Oslo)	Høst	615	37.9	46.8	10.9	4.4	30.4	8.3	61.6
Orroko Karafuto	Kishi	89	30.4	24.7	37.1	7.8	17.9	25.8	33.2
Osseta Caucasus	Jostrow	405	41.7	39.0	16.1	3.2	24.0	10.2	64.6
Permians	Parin	1 650	33.6	28.6	29.9	7.9	20.5	21.1	58.0
Poles	Halber and Mydlarski	11 488	52.5	37.6	20.9	9.0	28.2	11.1	62.6
Poles Warsaw	Amzel and Halber	2 928	33.7	38.4	19.4	8.5	26.9	15.5	57.9
Polynesians									
Hawaii	Nutt	413	36.5	60.8	2.2	0.5	38.2	1.8	60.4
Samoa	Stephenson	500	58.6	17.0	19.4	3.0	10.5	11.8	76.6
Tahiti	Shapiro	124	35.5	58.9	4.8	0.8	37.6	3.9	59.6
Tuamotus	Shapiro	176	48.3	51.7	0	0	31.5	0	69.5
Portuguese	Teague	500	35.2	52.8	8.2	3.6	34.4	6.5	59.4
Portuguese	Seuro de Cunha	459	38.4	52.5	6.1	3.0	33.3	4.7	61.9
Roumanians Wallachia	Manuila	1 521	33.7	43.1	15.6	7.4	29.8	12.3	58.1
Roumanians Transylvania	Popoviciu	2 376	36.6	40.9	14.5	8.0	28.5	12.0	60.5
Roumanians Wallachia	Popoviciu	1 278	33.5	41.2	19.0	6.5	27.5	13.6	57.9
Roumanians Moldavia	Jonescu	2 740	41.4	39.4	13.8	5.2	25.7	10.2	64.5
Russians Moscow	Avdeeva and Grunzev	2 200	32.0	38.5	23.0	6.5	25.9	16.1	56.6
Russians Leningrad	Grusdoff	510	32.0	34.0	26.0	8.0	24.8	18.8	56.6
Russians Odessa	Leitshik	1 831	31.8	46.2	17.4	4.6	29.8	11.9	56.5
Russians Tsimba	Wagner	1 000	36.2	52.7	22.9	7.7	22.7	16.7	60.7
Russians Tula	Konkoff	1 135	33.5	34.5	22.6	9.4	25.1	17.7	57.8
Russians Chelms	Melich and Grunot	1 356	35.7	52.4	22.2	9.2	23.8	17.5	59.8
Russians European	Rubashkin	10 151	33.9	36.7	22.7	6.7	25.4	16.0	58.2
Russians Asiatic	Rubashkin	11 130	55.5	51.5	24.4	8.6	22.6	17.5	59.6
Russians	All authors combined (after Streng)	57 122	32.9	35.6	23.2	8.1	25.1	17.1	5.4
Saxons Transylvania	Manuila	301	33.5	30.5	12.0	4.0	32.5	8.3	57.9
Scotch	Alexander	225	43.6	33.9	16.8	5.7	22.3	12.0	66.0
Senegalese	Juben	635	47.4	25.0	21.1	3.5	16.2	15.7	63.9
Serbs	L. and W. Altmann	500	38.8	47.8	23.8	4.8	28.8	10.8	57.8
Serbs (Yugoslavia)	Schmidt	899	35.6	38.2	21.2	5.0	24.6	14.1	60.0
Serbs Batschka	Schmidt	1 044	34.8	39.6	20.1	5.5	25.9	13.8	59.0
Slovaks	Trokan	9 837	36.0	36.2	19.4	8.3	25.6	15.1	60.0
Spanish Madrid	Garcia	296	44.1	46.5	5.4	3.7	29.8	4.9	66.4
Spanish	De Hoyos Saenz	1 035	43.6	51.2	3.9	1.1	31.1	2.6	66.0
Spanish (Barcelona)	Duran Jorda	22 120	41.5	45.6	9.4	3.3	28.8	6.9	64.3
Sumatrans									
Alas	Maasland and Appelmann	1 410	38.9	28.7	25.2	7.2	19.9	17.8	62.4
Mentawais	Johannsen and van Beukering	1 862	51.6	21.5	22.8	4.1	13.7	14.5	71.8
Niamnians	Maasland	4 401	2.2	5.7	21.8	0.4	3.1	11.8	84.9
Tobas	Hilfman and Maasland	2 000	45.2	18.8	31.2	4.8	12.6	20.0	67.2
Korintjis	van Lelyveld	285	24.6	26.6	39.0	9.8	20.3	30.2	49.6

TABLE 67 (Concluded)

Nationality	Investigator	Number of Persons Tested	Per Cent of Group				p	q	r
			O	A	B	AB			
Swabians Banat	Manuila	414	40.0	42.1	14.0	3.9	26.5	9.2	65.2
Swedes	Follegren	500	35.7	50.0	9.1	7.2	34.6	8.5	58.1
Swedes	Lindberger	500	33.5	51.0	10.0	5.5	54.1	8.1	57.9
Swedes, Stockholm	Hesser	533	36.9	46.9	9.7	6.4	51.0	8.5	60.7
Swedes	Wolff and Jonsson	4 260	37.9	46.7	10.5	5.1	30.5	8.0	61.6
Swiss Zurich	Clairmont	2 500	38.2	45.8	12.3	5.7	28.9	8.4	61.8
Swiss (Geneva)	Liengme and Goudet	4 000	40.2	48.9	7.9	3.0	30.9	5.9	65.4
Syria (villages)	Boyd and Boyd	300	30.3	39.0	17.1	4.6	25.8	12.4	62.8
Synans	Farr	509	41.4	26.3	24.0	5.3	17.5	15.9	66.5
Tatars Crimea	Bessudin	1 634	20.7	43.3	25.5	10.7	32.2	20.0	45.7
Tatars Kazan	Schwara and Niemo- vitkysa	500	27.8	30.0	28.8	15.4	24.7	24.0	52.8
Tatars Cheremiss	Petroff	550	32.6	25.3	35.7	6.4	17.2	24.1	57.1
Tibetans	Tennant	187	14.9	47.1	13.7	24.1	46.5	21.3	38.0
Tibetans (Bhutias)	Macfarlane	56	46.5	33.7	12.5	5.3	22.4	8.6	68.2
Transbaikial Asia	Werchoun	910	38.0	33.1	25.8	4.5	21.0	15.3	62.1
Tungus Evenki	Vuhnevsky	224	40.2	16.1	21.0	2	8.7	12.5	77.6
Tungus (Manchuria?)	Kunlun and Makino	813	28.2	29.1	33.1	8.6	22.7	25.4	53.0
Turkey (Istanbul)	Babacan	500	33.8	42.6	14.8	8.8	29.5	11.6	59.1
Turkey Yürük (nomads)	Irmak	400	51.3	40.5	6.2	2.0	24.2	4.3	71.8
Turkomans Turkistan	Mankiewicz	694	28.7	36.5	26.6	8.2	25.6	19.3	55.6
Turks Macedonia	L. and H. Herzfeld	500	36.8	38.0	18.6	6.6	25.6	13.6	60.7
Ukrainians Kherson	Schurak	665	56.4	35.6	19.7	8.3	25.2	15.1	60.3
Ukrainians Kiev	Tscherkassow Benass Atsein	673	27.6	41.2	22.1	9.1	29.5	1.0	52.5
Ukrainians Charkow	Hecker Karotkin Rosenberg	2 05	31.0	39.4	22.0	7.6	27.6	16.1	55.7
United States	Snyder	20 000	45.0	41.0	10.0	4.0	25.9	3	67.0
U.S. New York	Tiber	10 000	45.6	36.4	43.5	4.5	25.1	8.4	67.5
U.S. Detroit Mich	Culpepper and Ableson	5 000	44.5	36.1	14.3	5.2	23.2	10.2	65.7
U.S. Rochester Minn	Sandford	3 000	44.5	42.3	8.7	4.5	27.1	6.9	65.7
U.S. Brooklyn and New York	Wiener et al.	4 30	42.7	58.0	14.2	5.1	24.4	10.1	63.4
U.S. Utah	Matson and Schrader	985	47.0	42.0	9.7	1.2	24.7	5.0	68.6
Uzbek Chuvash A.S.S.R.	Vuhnevsky et al.	153	36.6	24.8	32.0	6.5	17.8	22.3	60.6
Uzbeks Turkistan	Mankiewicz	1 159	29.4	33.9	27.0	10.6	24.6	20.4	51.2
Welsh	Boyd and Boyd	192	47.9	32.8	16.2	3.1	20.6	10.8	69.2
Wotjaks (Finns)									
U.S.S.R.	Wagner	266	32.1	35.5	20.7	11.7	27.2	17.9	56.7
Wotjaks (Finns)									
U.S.S.R.	Parin	2 10	34.2	29.6	29.8	6.5	20.0	20.1	58.5

† This high frequency of groups AB makes one suspicious of errors in technic

used was not uniform. Therefore, with few exceptions, those results were excluded which failed to satisfy the Bernstein equation. For convenience of reference the results are arranged alphabetically according to the nationalities.

When comparing the distribution of the blood groups in two different populations, it must be remembered that, of course, the reliability of the statistics depends upon the number of individuals examined. The formula for the probable error of a frequency is given on page 170. For convenience a table of probable errors is given here (cf. table 68).

The probable error principle is applied as follows. Suppose that the distribution of the blood groups is being studied in two different races, the sample of the population examined in each case consisting of 100 individuals. In one sample the frequency

TABLE 68
PROBABLE ERRORS (Per Cent)

Size of Sample	Observed Frequencies (Per Cent)				
	10 or 90	20 or 80	30 or 70	40 or 60	50
100	2.02	2.69	3.09	3.31	3.37
200	1.43	1.91	2.19	2.34	2.38
300	1.17	1.56	1.78	1.92	1.93
400	1.01	1.35	1.59	1.65	1.68
500	0.91	1.21	1.38	1.49	1.52
750	0.74	0.99	1.14	1.20	1.23
1 000	0.64	0.85	0.98	1.05	1.07
1 500	0.52	0.69	0.80	0.85	0.87
2 000	0.45	0.60	0.69	0.73	0.75
3 000	0.37	0.49	0.57	0.60	0.62
5 000	0.29	0.39	0.44	0.47	0.48
10 000	0.20	0.27	0.31	0.32	0.33
20 000	0.14	0.19	0.22	0.23	0.23

of group O is found to be 50 per cent and in the other 60 per cent and it is desired to know whether the difference of 10 per cent is significant. If the probable errors (equal to p_1 and p_2 respectively) of two independent observed frequencies are known, the probable error of the difference between the observed frequencies is calculated as follows:²

$$p = \sqrt{p_1^2 + p_2^2}$$

In the present case

$$p_1 = 3.37 \text{ per cent}$$

$$p_2 = 3.31 \text{ per cent}$$

$$\text{so that } p = 4.82 \text{ per cent}$$

$$\text{and } \frac{\text{Dev}}{\text{P.E.}} = \frac{10}{4.8} = 2.1$$

Since the deviation is less than three times the probable error, it need not be significant (cf. page 170). On the other hand, if the size of each of the samples examined was 400 individuals, a deviation of 10 per cent would be considered significant, since the probable errors would be half as large. In this case

$$\frac{\text{Dev}}{\text{P.E.}} = \frac{10}{2.4} = 4.2$$

The most striking way to show the relation of the various races to one another with respect to the blood groups is to plot the frequencies

² See Pearl, *Medical Biometrics and Statistics*, 370 pp., W. B. Saunders Co. (1927); Kelley, *Statistical Method*, 385 pp., Macmillan Co., New York (1924), etc.

of the genes on a graph. For the reason pointed out above, only the frequencies of two of the genes need be plotted. Thus, the frequencies of the gene A (p) and gene B (q) for the various races can be represented by points on a plane by rectangular coordinates.

The same principle has been conveniently applied by dividing a plane into squares, each square having sides five units in length (Snyder³). Populations falling within the same square are listed together. The data of table 67 are presented in this graphic manner in table 69. The relationships of the races to one another are then readily recognized. It must be remembered, however, that the differences may be appreciably smaller between two races in adjacent rectangles than between two races in the same one, because of the comparatively large size of the rectangles. If it is desired to determine whether or not two populations differ significantly in their serological constitution, it will be necessary to compare the differences between the group frequencies with their probable errors.

A rather neat way of representing the distribution of the blood groups in a population has been suggested by Streng,⁴ by applying the theorem that in an equilateral triangle, the sum of the distances of any point within the triangle from the three sides is equal to the altitude. If the length of the altitude is taken equal to unity (or 100 per cent) and the

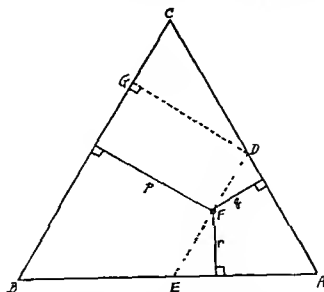


FIG. 60. STRENG'S TRIANGLE

³ *Amer Jour Phys Anthropol* 9: 233 (1926), *Human Biology* 2: 128 (1930)

⁴ *Acta Soc med Fenn Duodecim* 8: 1 (1926), *Acta Pathol et Microbiol Scand* Suppl. V, p. 59 (1930)

three distances equal to p , q , and r , respectively, then $p+q+r=1$, or 100 per cent. This method has the advantage that the frequencies of all three genes are represented.

The theorem upon which Streng's method is based is proved as follows. If AB , BC , and CA are the sides of an equilateral triangle (cf. fig. 60) and if the sines of the angles which are of course each equal to 60° , are represented by s then since the altitude is taken equal in length to unity

$$\overline{AB}s = \overline{BC}s = \overline{CA}s = 1$$

Through F draw line \overline{DE} parallel to \overline{BC}

Then $\triangle DEA$ is an equilateral triangle

Draw \overline{DG} perpendicular to \overline{BC}

Then $\overline{DG} = p = \overline{CD}s$

$$q = \overline{DF}s$$

$$\text{and } r = \overline{EF}s$$

$$q+r = (\overline{DF} + \overline{FE})s = \overline{DE}s = \overline{DA}s$$

$$\text{and } p+q+r = (\overline{CD} + \overline{DA})s = \overline{AC}s$$

$$\text{so that } p+q+r=1$$

Streng has pointed out that this principle may also be applied by representing the altitudes of the equilateral triangle by three lines (each measuring 100 units in length) intersecting one another at 60° angles to form a system of three coordinate axes. The point of intersection of these lines need not be fixed, provided that the sum of the distances of this point from the ends of the axes is equal to 100 (cf. fig. 61). By thus shifting the axes a particular area on the plane can be more conveniently represented. A similar procedure can also be used of course with rectangular coordinates. The graphic representation is not fundamentally different with rectangular and triangular coordinates, but the former may be preferable because of their greater simplicity.

Ottenberg³ proposed that on the basis of the frequencies of the properties O , A , and B six main racial types could be distinguished listed in table 70. According to

TABLE 70

Types		O	A	B
I	European	39	43	12
II	Intermediate (Arabs, Turks, Russians, etc.)	40	33	20
III	Hunan (Japan, South China, Hungary, etc.)	28	39	19
IV	Hindo-Manchu (Korea, North China, Gypsies, Hindus)	30	19	39
V	Afro-South Asiatic (Negroes, Madagascans, Malaysians)	42	24	28
VI	Pacific American (Indians, Australians, Filipinos, Icelanders)	67	29	3

³ *Jour. Amer. Med. Assoc.* 84: 1393 (1925)

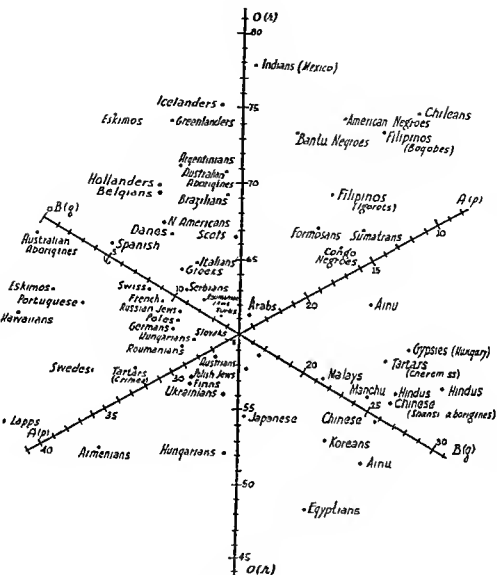


FIG. 61 SEROLOGICAL COMPOSITION OF MANY OF THE PEOPLES OF THE WORLD
(Represented by Means of Triangular Coordinates)

Snyder, the Pacific American type should be subdivided and the Australians placed in a separate type. Obviously, the subdivision of races into types by this or any similar method is arbitrary.

The application of blood grouping in anthropology is based upon the hereditary nature of the blood groups, and the fact that there can be no conscious selection of mates dependent upon the blood groups. After the population has reached equilibrium, therefore, the proportions of

the blood groups remain constant from generation to generation, provided there is no crossing with other peoples. As an illustration of this principle may be cited the observations of Vêrzar and Weszczky.⁶ These workers studied the distribution of the blood groups in gypsies (cf. table 71) who had originally lived in India, and then migrated to Hungary where they lived for several hundred years. It can be seen that the serological composition of the gypsies does not differ significantly from that of the natives of India, although it differs greatly from that of the Hungarians among whom they lived. This confirms the observa-

TABLE 71

ILLUSTRATING THE CONSTANCY OF THE DISTRIBUTION OF THE BLOOD GROUPS IN THE ABSENCE OF RACIAL ADMIXTURE

Race	Number of Persons	Per Cent in Group			
		O	A	B	AB
Gypsies (in Hungary)	385	34.2 ± 1.6	21.1 ± 1.4	38.4 ± 1.4	8.5 ± 1.0
Hindus	1000	31.3 ± 1.0	19.0 ± 0.8	41.2 ± 1.1	8.5 ± 0.5
Difference		2.9 ± 1.9	2.1 ± 1.6	2.8 ± 1.8	0.0 ± 1.1
Hungarians	1500	31.0 ± 0.8	38.0 ± 0.9	18.8 ± 0.7	12.2 ± 0.6
Difference		3.2 ± 1.8	16.9 ± 1.7	19.6 ± 1.6	3.7 ± 1.2
German (Colonies in Hungary)	476	40.8 ± 1.5	43.5 ± 1.5	12.6 ± 1.0	3.1 ± 0.5
Germans (Heidelberg)	500	40.0 ± 1.5	43.0 ± 1.5	12.0 ± 1.0	5.0 ± 0.7
Difference		0.8 ± 2.1	0.5 ± 2.1	0.6 ± 1.4	1.9 ± 0.8
Hungarians	1500	31.0 ± 0.8	38.0 ± 0.9	18.8 ± 0.7	12.2 ± 0.6
Difference		9.8 ± 1.7	5.5 ± 1.8	6.2 ± 1.3	9.1 ± 0.8

tion that little intermarriage took place between the gypsies in Hungary and other races. Vêrzar and Weszczky also studied the distribution of the blood groups among German colonies in Hungary, started by German settlers who migrated into that country at the beginning of the eighteenth century. These people, who retained their original language and customs, still have the blood group distribution of the German people.

The effect of racial crossing on the distribution of the blood groups in the American Indians is illustrated in table 72. Similarly, the Jamaicans, who have undergone frequent crossing with white peoples, exhibit a

⁶ *Biochem Ztschr* 126: 33 (1921)

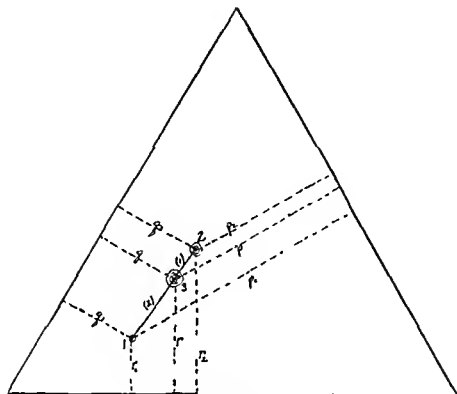


FIG 62 DIAGRAMMATIC REPRESENTATION OF MIXTURE OF RACES
IN STRENG'S TRIANGLE
(After Bernstein)

TABLE 72
GRADED SERIES SHOWING THE EFFECT OF CROSSING ON THE
PROPORTIONS OF THE GROUPS
(After Snyder)

Race	Number Studied	Per Cent in Group				p	q	r
		O	A	B	AB			
Indians supposed to be pure	453	91.3	7.7	1.0	0.0	3.9	0.5	95.5
All Indians (mixed and pure)	1134	79.1	16.4	3.4	0.9	9.2	2.3	88.9
Indians known to be mixed	409	64.8	25.6	7.1	2.4	15.3	4.9	80.5
Americans (White)	1000	45.0	42.0	10.0	3.0	25.8	6.7	67.1

blood group distribution definitely nearer to that of the whites than the Yoruba, a tribe in southern Nigeria from which the Jamaican arose. In Berlin and Leipzig the frequency of gene *B* is higher than in the western part of Germany because of the larger Slav element. Wiechmann and Paal⁷ found that the frequencies of the blood groups differed in Cologne and the adjacent country, and they attributed this to the more frequent mixing of races in large cities.

Scattered groups of the same people may show statistically significant and even wide variations in the distributions of the blood groups. The Polynesians and Filipinos, groups of whom are located on different islands, illustrate this point very well. This apparent anomaly has been explained by the effect of isolation and intermarriage between close blood relations.

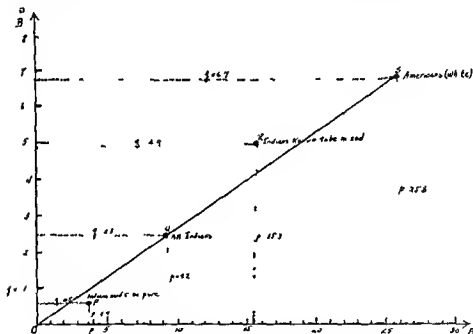


FIG. 63 GRAPHIC REPRESENTATION OF BLOOD GROUP DISTRIBUTIONS OF THE GRADED SERIES OF INDIANS AND WHITES OF TABLE 72

Bernstein⁸ has pointed out that if it is known that a certain population has resulted from crosses between two races it is possible to predict the blood group frequencies in this population if the blood group frequencies in the original races are known and if the proportion of individuals of each race that entered the cross is known. This is done as follows. If P and Q are points on a plane representing the blood group compositions of the two original races (cf fig 62) the point (M)

¹ *Münch med Woch* 73 606 (1926), p 2202 (1926)

* *Comitato Italiano per lo Studio dei Problemi della Popolazione* Istituto Poligrafico dello Stato Roma (1932)

representing the mixed population lies on a line joining these points. Furthermore the distances of the point M from P and Q are inversely proportional to the number of individuals of the corresponding races that entered the cross. As an illustration let us analyze the graded series of white and Indian populations given in table 72. The respective populations are represented by the points P, Q, R, and S in figure 63. The scales for p and q are different in order that the graph may be easier to read. For this reason the deviations of points P, Q, and R from line OS seem greater than they really are.

Since the distance between any two points having the coordinates (p_1, q_1) and

$$(p_2, q_2) \text{ respectively} = \sqrt{(p_1 - p_2)^2 + (q_1 - q_2)^2}$$

$$OS = \sqrt{(25.8)^2 + (6.7)^2} = 26.66$$

$$OP = \sqrt{(3.9)^2 + (0.5)^2} = 3.93$$

$$OQ = \sqrt{(9.2)^2 + (2.3)^2} = 9.48$$

$$OR = \sqrt{(15.3)^2 + (4.9)^2} = 16.07$$

$$PS = \sqrt{(21.9)^2 + (6.2)^2} = 22.7$$

$$QS = \sqrt{(16.6)^2 + (4.5)^2} = 17.20$$

$$RS = \sqrt{(10.5)^2 + (1.8)^2} = 10.63$$

Since $OP + PS = OQ + QS = OR + RS$, $OS = 26.7$ approximately, the points P, Q, R must lie very close to line OS. It is therefore reasonable to conclude that the Indian tribes studied by Snyder originally had a composition approximating that represented by point O, i.e. $p = 0$, $q = 0$, $r = 100$. The populations P, Q, and R represent mixtures of Indians and whites. The percentage of white blood in these various populations is calculated as follows:

$$\text{For population P} = \frac{OP}{OS} = \frac{3.9}{26.7} = 14.6 \text{ per cent white}$$

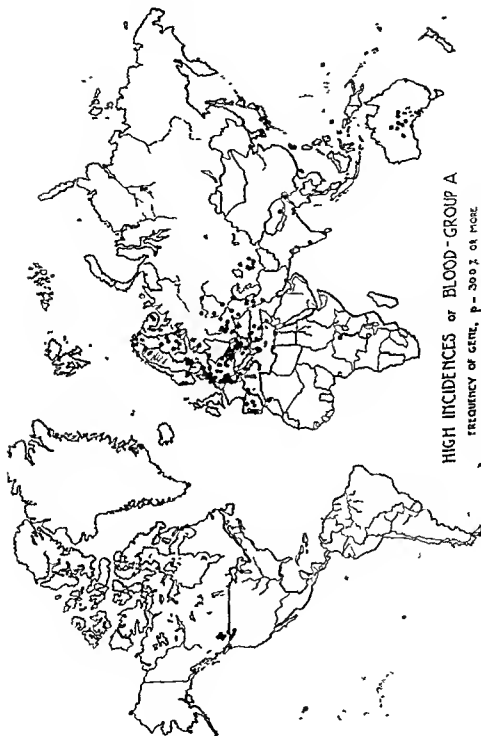
$$\text{For population Q} = \frac{OQ}{OS} = \frac{9.5}{26.7} = 35.6 \text{ per cent white}$$

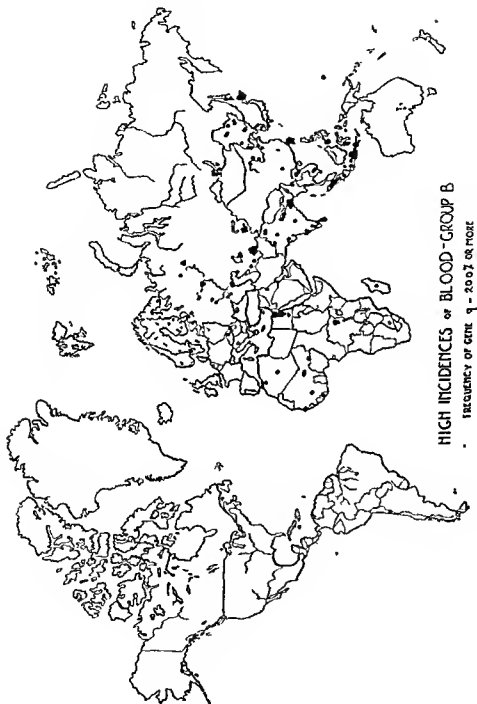
$$\text{and population R} = \frac{OR}{OS} = \frac{16.1}{26.7} = 60.3 \text{ per cent white}$$

The theory that pure Indians belong to group O has been satisfied in several cases by actual observations. Thus 200 full blooded Indians in Peru were all found to belong to group O, whereas only 75.1 per cent of the half breeds examined belonged to that group (cf. table 67). However, exceptions to this rule have been reported particularly by Matson and Schrader* who determined the blood groups of Indians of the Blackfeet and Blood tribes (in the U.S. and Canada) and found as high as 75 per cent group A (cf. table 67).

Geographical Distribution of the Blood Group Genes. A convenient way of summarizing the results of the blood group investigations is to plot the data on maps. The most common procedure is to connect points of equal gene frequencies by lines called 'isogenes' so as to produce the effect of contour maps. This method has the disadvantages that because of incomplete information sufficient points cannot be plotted to draw the lines accurately, and more important, due to marked local fluctuations caused by various factors (e.g., in large cities, etc.) a con-

* *Jour Immunol* 25: 155 (1933). Another exception was reported by Golden [*Lancet* 2: 218 (1930)] who found a tribe of Indians with a high frequency of group B (cf. table 67). Also see Boyd *Amer Jour Phys Anthropol* 25: 215 (1939).





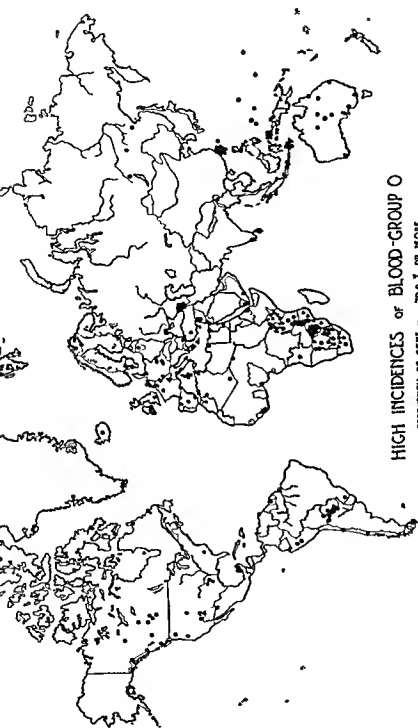


FIG. 64 GEOGRAPHICAL DISTRIBUTION OF THE BLOOD GROUP GENES (After Candela)

Each dot represents the result of a separate investigation. In order to bring out the characteristic blood group distribution only those series have been plotted which show particularly high frequencies of the respective genes. The most striking picture was obtained by selecting the frequencies indicated in the charts: $p \geq 30$ per cent, $q \geq 20$ and $r \geq 70$.

fusing picture is obtained, concealing the major trend of the findings

Candela has overcome some of these difficulties by plotting on three maps populations with high frequencies of genes *A*, *B* and *O*, respectively, and his maps are reproduced in fig. 64. Each point on the maps represents the result of a separate investigation, such as those listed in table 67. In the Americas only the frequencies of genes for American Indians have been plotted, and in Australia only the results for the aborigines. The gene frequencies of white populations in these continents, and also in Africa and Asia naturally correspond to those of the European countries from which they are derived. From the density or concentration of dots one gains the impression of the presence of certain foci at which the frequencies of the genes are highest. It can be seen from the first map that there are numerous populations in Europe with high frequencies of gene *A*, a smaller number is present in North America (Blackfeet and Blood tribes of American Indians and Eskimos), Australia (aborigines) and on the east coast of Asia while only scattered populations exist with high frequencies of gene *A* in Africa. The second map shows that most of the populations with the highest frequencies of gene *B* are located in Asia, North and Central Africa and Eastern Europe. The third map demonstrates that the foci with high frequency of gene *O* are located mainly in the western hemisphere (Indians and Eskimos), in the negroid peoples of Africa, and in Europe and Australia, while such populations are less frequent in the parts of Asia so far examined.

Theories Concerning the Origin of the Blood Groups The manner in which the varying blood group distributions in the different peoples of the world arose is not known. Several theories have been suggested none of which has been definitely established. For example, it has been suggested that the predominance of group *O* in practically all races and its occurrence in a practically pure state in American Indians is evidence that in man only the property *O* existed originally, and that the properties *A* and *B* appeared at a later date by mutation.¹⁰ Then the fact that the properties *A* and *B* are practically absent in most Indian races would suggest that the separation of these races from the main Asiatic trunk occurred before the appearance of agglutinogens *A* and *B*. This theory must be modified, however, in the light of the observations on "Blackfeet" and "Blood" Indians (cf. page 312). The predominance of factor *A* in western Europe has been considered an indication that the factor first appeared in that locality of the world and then gradually spread eastwards, and similarly, the predominance of factor *B* in Asiatic peoples has been considered evidence of the origin of property *B* in Asia.

Objections to the theory that race *O* was the original race and that

¹⁰ Bernstein *Ztschr f indukt Abstamm u Vererbungslehre* 37: 237 (1925) 56 233 (1930), Snyder, *loc cit*

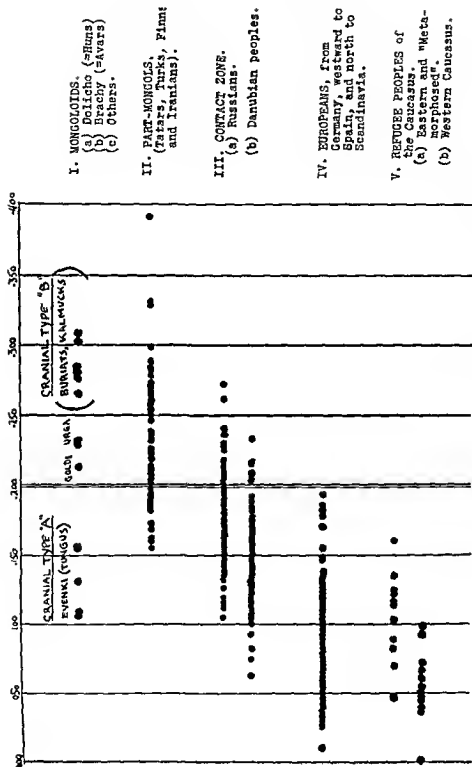


FIG 65 THE VALUES OF Q IN FIVE CATEGORIES OF POPULATIONS (After Candela)

factors A and B appeared later have been raised by some authors because of the occurrence of the agglutinogens A and B in the anthropoid apes (cf page 332). According to Snyder, the most likely explanation is that the mutations arose independently in apes and in man. Some workers¹¹ have suggested that instead of a single original O race there were three (or more) races in which the genes *O*, *A*, and *B*, respectively, predominated.¹² The present distributions of the blood groups are supposed to have resulted from migration and crossing of these original races (cf page 337).

Some other observations on the geographical distribution of the blood groups in Europe and Asia have been adduced as evidence for further hypotheses on the problem. Factor A, though decreasing from west to east, seldom becomes rare whereas factor B becomes quite rare in western peoples, the frequency of gene *B* (*q*) in some regions being as low as 3 to 5 per cent. In addition, there is a second point of maximum frequency for factor A in southern Korea and southern Japan but this maximum is not as high as the one existing in western Europe. The following explanations have been suggested for these observations:

1 Mutation B occurred more recently than mutation A or mutation B originally occurred in fewer people than mutation A.

2 An independent mutation of factor A occurred in Asia.

Which if any of the theories mentioned is correct cannot be said. However if the blood group distributions in Europe at the present time are correlated with the historical facts and other anthropological data some conclusions can be drawn. As an example the well known gradient in the frequency of gene *B* as one proceeds from East to West (cf page 296) suggests as already mentioned that this gene was introduced into Europe from Asia. This idea is further supported by an analysis made by Candela (cf fig 65) and by the isogene maps constructed by Haldane.¹³

Haldane points out that the steepest gradient runs along the continental boundary of Scandinavia through the Baltic Sea and central Europe and this he explains as due to the greater ease of migration over the steppes of Russia, the Ukraine and the North Sea then over the mountains and through the forests of central Europe. The sharpest drop in the frequency of the *B* gene occurs between Russia and Scandinavia.

According to Candela¹⁴ the data plotted in figure 65 together with racial and historical data indicate that the gene *B* was introduced into Europe through the agency of the Mongolian invasions occurring between the 5th and the 15th centuries.

¹ Bernstein *loc cit* Cf Landsteiner and Miller *Jour Exp Med* 43 853 (1925)

¹² Another view supposing that the original stocks were characterized not necessarily by a single blood group but by particular blood group distributions is held by Candela (personal communication) [Cf Schiff and Boyd *Blood Grouping Technique* p 207 (1942)]

¹³ *Human Biology* 12 457 (1940), cf Coon *The Races of Europe* Macmillan Co (1939)

¹⁴ *Human Biology* 14 413 (1942)

He believes that the gene was derived from the brachycephalic central Asiatic Mongols best represented at the present time by the Buriats and Kalmucks

Of the two possibilities (1) that the blood groups always existed in man, i.e., that they are older than the present races, or (2) that the blood groups arose at a later period in man's development, the former is favored by Wyman and Boyd¹⁵ and by Fisher.¹⁶ Wyman and Boyd suggest that in speculating about this question one should go back to the earliest days of mankind, when the total number of men in the world was very small. It is conceivable that small groups (families) became isolated and multiplied to a considerable degree. If the first men had A, B and O, then it would be possible as a matter of pure chance that these original families were different as to the blood groups and gave rise to certain primitive stocks, each belonging chiefly to one or another of the groups.

This hypothesis would explain why races widely separated geographically closely resemble each other with regard to the distribution of the blood groups. A few striking examples are the similarities between Greenlanders and Australians and between the Chinese of Canton and the Negroes of South Belgian Congo (cf. table 67). Wyman and Boyd believe that such similarities can be explained by assuming early dispersions of the blood group genes from certain centers. It could then be imagined that the stock from which the Eskimos arose and another the precursors of Australians may have received similar amounts of A and B, and carried them to distant regions.

Those not wishing to concede the antiquity of the blood groups prefer to explain the cases of paradoxical similarities by postulating later independent mutations. Fisher has shown that unless mutations of genes like the blood group genes which have no advantage in natural selection, continue to occur at a certain rate they would tend to die out after only comparatively few generations. The rate of spread of a character with no selective value and which mutates repeatedly is given by the formula (Fisher)

$$q = e^{-\mu n}$$

where q = proportion of unmutated genes n = number of generations elapsing μ = fraction of the total number of genes mutating each generation. Using this formula and assuming a mutation frequency¹⁷ of 10^{-8} , Wyman and Boyd have estimated that it would require 745 000 years for the genes A and B to attain their present maximum frequencies. To account for a build-up of the observed frequencies of A

¹⁵ *Amer Anthropol* 37: 181 (1935) 39: 583 (1937)

¹⁶ Quoted after Wyman and Boyd

¹⁷ That is once in a 10^8 life-cycles. This figure was chosen on the basis of calculations of the mutation rate for hemophilia in man. Cf. Haldane *Human Biology* 12: 457 (1940)

and B in Asia since the migrations of man to America (on the assumption that this took place some 50,000 years ago), it would be necessary to postulate a mutation rate of 10^{-4} (in a population of 1 000,000 people, this would imply about 5 or 6 mutations each to A and B every year) Wyman and Boyd consider this rate too high to be probable and in addition point out that there seems to be no reason why mutation at a similar rate should not have gone on in America at the same time. Therefore in the opinion of these authors, the mutation theory should be discarded unless later work demonstrates some selective value for the blood groups, or for factors closely linked to them.

On the other hand, this argument does not hold if the opinion of Gates¹⁸ is correct that there are periods during which particular mutations occur at a greatly increased rate. Direct evidence to support this idea as applied to the blood group factors is not available of course. *Blood Groups and Racial Origins*. The application of blood grouping in anthropology has been complemented by the development of technics for examining mummified material and skeletal remains. This is exemplified by the results of tests on the skeletons of 30 prehistoric Aleutian mummies.¹⁹ The Aleuts were generally believed to have arisen from an Eskimo-Indian cross, however, the findings of groups B and AB in 8 of 30 mummies¹⁹ rendered this theory untenable. As in some other human stocks among pure Eskimos and North American Indians the incidence of the B factor is very low, in Asia however B is common. The conclusion, based on these data that the Aleuts were of Asiatic origin, has been supported by information from other branches of anthropology.

That the grouping of skeletal remains is a difficult technical problem is self-evident but as has been demonstrated by Candela²⁰ it is nevertheless possible to get reliable results with such material.

While in principle the tests do not differ from the procedures used for identifying the agglutinogens in blood stains (cf page 409) in practice the procedure is complicated by the fact that ground bone has a high degree of non specific adsorptive power. This makes it essential to start with A and B test sera of exactly equal strength since if the sera are not balanced or if they become unequal through deterioration the non specific reduction in titer of the weaker one may lead to a false A or B diagnosis.

Accordingly the preliminary titration of the test sera is one of the most important steps.

Candela's success in grouping skeletal remains probably depended upon the following modifications in the technic. Firstly he varied the proportion between cells and serum in the titrations and tests by using a large volume of serum and only a

¹⁸ Gates *Genetica* 18 47 (1936)

¹⁹ Candela *Amer Jour Phys Anthropol* 24 361 (1939)

²⁰ Candela *Amer Jour Phys Anthropol* 21 429 (1936) *ibid* 23 71 (1937) *ibid* 24 361 (1939), *ibid* 25 187 (1939), *ibid* 27 365 (1940) *Amer Antiquity* 5 No 1 (1939)

small amount of cells (cf page 20) In this way the test sera could be used in higher dilutions in the absorption tests (cf page 284) Secondly when tests were made for agglutnogen A test cells of subgroup A₁ were always used Finally the absorptions were carried out in the cold over an extended period (48 hours) It should also be mentioned that the reading of the reactions may be complicated by hemolysis of the test cells caused by acidity or the presence of glycerol in the marrow but Candela found that the addition of small amounts of saturated salt solution at times together with a few drops of N/50 alkali may obviate this difficulty Obviously this procedure may also prove useful in tests on other similar material For further details the reader is referred to the original papers

The success in the grouping of skeletal remains by the absorption technic probably depends on the presence of dried marrow and blood on the lining of the cancellous bone used for the examination

Mummified tissue had previously been used for grouping tests by Boyd and Boyd After first demonstrating that preserved muscle tissue can be grouped by the absorption technic,²¹ Boyd and Boyd²² applied this procedure to mummies In their initial experiments, 8 out of 10 American Indian specimens failed to absorb either anti A or anti B agglutinins while 2 absorbed anti A but not anti B agglutinins On the other hand, while 3 of 6 Egyptian specimens exhibited no group specific properties, the other three gave reactions corresponding to group B Boyd and Boyd²³ then extended their studies to include 300 mummies, and the results of these investigations served to amplify and confirm their previous findings As was pointed out by Matson,²⁴ mummified tissue can also be grouped by preparing saline extracts and then testing these by the inhibition technic (cf page 278) For the identification of group O tissues, Matson used anti O ox sera, and he found the O antigen to be present in 15 of 16 American Indian mummies he examined On the other hand 2 out of 6 Egyptian mummies possessed B antigen and one contained A

When the absorption method is used the presence of preservatives in some samples may interfere with the reactions Extracts prepared by freezing and thawing tissue as advocated by Matson are apt to be weak Since the water soluble group substances are heat stable the extractions can be made with hot water (cf page 416) these extracts being evaporated to dryness and the residue re dissolved in a small volume of saline solution If large amounts of material are available for examination concentrated extracts can be obtained in this manner which are suitable for examination by the inhibition technic²⁵

Relationship Between Blood Groups and Anthropological Characters

Studies on the correlation between blood groups and other constitutional characters have failed to convincingly establish any relationship between

²¹ Boyd and Boyd *Science* 78 578 (1933) *Jour Immunol* 26 489 (1934)

²² *Proc Soc Exp Biol and Med* 31 671 (1934)

²³ *Jour Immunol* 32 307 (1937)

²⁴ *Jour Immunol* 30 459 (1936)

²⁵ Cf Candela *Science News Letter* 39 105 (1941)

the blood properties and such characters as size, weight, color of the skin and hair, etc. For example, in the some population, the distribution of the blood groups is the same among people with light hair as among people with dark hair

A number of authors have claimed to have found some correlation between the blood groups and certain constitutional characters. In some of these studies the series of cases examined was small and the differences found were really not significant as the authors believed. This can be demonstrated by calculating the probable errors of the observed frequencies (cf. page 303). In a number of cases populations in large cities were studied so that the conclusions are invalid because there may have been recent admixture of different races. Thus if a race of people having blue eyes and characterized by a high frequency of group A is mixed with a race of people having brown eyes and a high frequency of group B in the mixed population the frequency of group A will be higher among blue-eyed individuals and the frequency of group B will be higher among brown-eyed individuals. This correlation between color of the eyes and blood groups is only temporary, since with random intermarriage the distribution of the blood groups will become identical among blue-eyed and brown-eyed individuals. On the other hand if races in the population do not intermarry the correlation between blood groups and eye-color will persist. This would not mean, however, that there is any intrinsic connection between the two traits. As Streng has shown this explanation seems to apply to the apparent correlation between blood groups and shape of the head which has been reported by Klein and Osthoff.*

RACIAL DISTRIBUTION OF THE SUBGROUPS OF GROUPS A AND AB

The first studies on the distribution of the subgroups were carried out in the U S¹¹ and in England¹² and revealed that in white individuals of groups A and AB about 80 per cent belong to subgroups A₁ and A₂B. Among negroes living in New York City, Landsteiner and Levine¹³ found that the relative incidence of subgroup A₂ in groups A and AB was higher than in white persons. On the other hand Nigg¹⁴ found that in pure Hawaiians, who are characterized by a high incidence of group A (60.8 per cent), all the group A individuals examined belonged to subgroup A₁. Of interest are also the studies of Matson, Levine and Schrader¹⁵ on the distribution of the subgroups of A in Blackfoot Indians, a tribe with even higher frequency of group A than the Hawaiians (cf. page 312). Of 93 putative full blooded Indians tested only one was found to belong to subgroup A. More recently the studies have been extended to include other races but the number of investigations on the subgroups is still relatively small.

* *Arch f Rassenbiol* 17: 371 (1926)

¹¹ Coca and Klein *Jour Immunol* 8: 487 (1923) Kline, Ecker and Young *Jour Immunol* 10: 595 (1925)

¹² *Sm on Jour Path and Bact* 29: 279 (1926)

¹³ *Jour Immunol* 16: 123 (1929)

¹⁴ *Jour Immunol* 19: 93 (1930)

¹⁵ *Proc Soc Exp Biol and Med* 35: 46 (1936)

TABLE 73
RACIAL DISTRIBUTION OF THE SUBGROUPS OF GROUPS A AND AB*

Population	Investigators	Number of Persons Tested	Per Cent of Groups and Subgroups					Index A_1+A_2B A_1+A_1B	Frequencies of genes			
			O	A_1	A_2	B	A_1B	A_2B	p_1	p_2	q	r
American Indians (full blood), Tacoma, Wash	Landsteiner, Wiener and Matson	120	73.3	25.8	0	0.8	0	0	13.9	0	0.5	85.6
American Indians (not pure)		155	58.1	31.6	3.2	4.5	2.6	0	0.09	2.1	2.9	76.2
Denmark	Wellisch and Thomsen	390	41.5	35.6	10.8	8.2	2.6	1.3	0.37	21.5	6.1	64.5
Denmark	Clausen	1 853	40.0	32.7	9.8	12.4	2.8	2.3	0.34	20.2	7.3	63.3
Egypt (Cairo)	Matta	516	26.6	29.1	6.6	27.1	7.0	3.5	0.28	21.3	6.0	51.6
English (Sheffield)	Simson	421	34.0	43.2	10.2	10.0	2.1	0.5	0.23	27.0	8.2	58.3
English (Shenley, Hertfordshire)	Thomas and Hewitt	900	42.1	35.8	8.0	10.1	3.1	0.9	0.23	21.9	5.9	64.9
English (London)	Taylor and Prior	345	46.4	35.9	7.5	8.4	1.2	0.6	0.22	21.2	5.9	68.1
Finland	Mustakallio	7,120	33.9	32.3	10.7	15.8	4.4	2.9	0.37	20.1	9.3	58.2
Germany	Dahr, Offe and Weber	416	40.1	37.5	7.0	11.3	3.3	0.8	0.19	23.4	5.3	63.3
Hawaii	Nigg	413	36.5	60.8	0	2.2	0.5	0	0	38.2	0	60.4
Russia	Blinov	763	33.7	30.8	7.6	20.8	4.0	3.1	0.31	20.6	6.2	58.1
Sweden	Wolff and Jonsson	1,200	37.9	36.9	9.8	10.3	3.9	1.2	0.27	22.9	8.5	61.6
USA (Whites, N.Y.)	Wiener and Sonnet	1,077	41.7	29.0	8.9	13.9	5.2	1.4	0.30	18.1	6.5	64.6
USA (Negroes, N.Y.)	Wiener	189	48.1	19.6	6.8	22.8	1.6	1.1	0.37	12.2	4.8	69.4

* This table includes only reports containing data which permitted calculation of the frequencies of genes A_1 , B_1 , B_2 , B and O . The remainder of the studies on the distribution of the subgroups are presented in table 74.

† These results include 243 parents from the paper by Wiener and Rothberg [*Human Biology* 5: 577 (1933)] and 834 whites from a random series (unpublished).

The available data on the racial distribution of the subgroups are summarized in two tables (tables 73 and 74). In the first table are included those studies in which data as to the incidence of all groups and subgroups were presented making possible the computation of the frequencies of the four genes A_1 , A_2 , B and O . The second table contains studies which supply information only with regard to the relative incidence of the subgroups represented by the index A_2/A_1 . Obviously and as is seen from table 74 the fact that the index A_2/A_1 in a given population is high does not necessarily indicate a high absolute frequency for subgroup A .

It is evident that the tests for the subgroups furnish another tool

TABLE 74
RACIAL DISTRIBUTION OF THE SUBGROUPS OF A^*

People	Place	Investigator	Number Tested	A_2/A_1
American Indians	Montana	Matson Levine and Schrader	93	0.01
Basques	San Sebastian, Spain	Boyd and Boyd	91	0.39
Denmark	Copenhagen	Worsaae	197	0.25
Egypt	Assiut	Boyd and Boyd	525	0.20
Egypt	Cairo	Boyd and Boyd	613	0.24
Egypt	Cairo	Moharram	500	0.17
Eskimos	Labrador Baffin Is.	Sewell (after Boyd)	199	very small
Germany	Berlin	Akune	704	0.19
Germany	Heidelberg	Kloppstock	523	0.23
Germany	Cologne	Dahr	1,293	0.20
Germany	Cologne	Muller	423	0.23
Ireland	Dublin	Boyd and Boyd	399	0.46
Russia	Kharkov	Boyd and Boyd	310	0.29
Russia	Zagorsk	Boyd and Boyd	489	0.26
U S A (whites)	New York City	Landsteiner and Levine	119	0.16
U S A (negroes)	New York City	Landsteiner and Levine	89	0.67
Wales	North Wales	Boyd and Boyd	192	0.53

* This table includes studies with information only with regard to the relative incidence of the subgroups. Most of these data are cited after Boyd [*Tabulae Biologicae*, Vol XVII part 2 (1939)]

to the anthropologist for studying racial relationships and racial origins. For example, the complete or almost complete absence of subgroups A_2 among American Indians, Polynesians and Eskimos even in tribes with very high frequencies of group A is in conformity with other anthropological data indicating a certain degree of relationship between these races.

RACIAL DISTRIBUTION OF THE AGGLUTINOGENS M AND N

The first studies on the racial distribution of the agglutinogens M and N were made by Landsteiner and Levine³² These authors found that there were significant differences between whites and American Indians with regard to the distribution of these properties During the past decade the distribution of M and N in other races has been studied and the results of these investigations are summarized in table 75. In preparing this table, those studies were excluded whose reliability is doubtful because the value of D was more than three times greater than σ_D (cf page 239)

Many of the races thus far examined have the same or similar distributions of the properties M and N The most striking exceptions are the American Indians, the Eskimos, the Hindus, the Bedouin, the Australian aborigines and the Ainu The distributions of M and N in the Finns and Swedes also deviate significantly from those of the other races, but here the differences are less striking

Since $m+n=1$, and from the relations $m=M+\frac{MN}{2}$ and $n=N+\frac{MN}{2}$ (cf page 237), it follows that if the frequency of either gene or of any of the three types is known, the distribution of the agglutinogens M and N in the population can be calculated Thus, the serological composition of the American Indians is fully characterized (so far as M and N go that is) by the frequency $m=77.6$

From these considerations, it is evident that the distribution of M and N in various races can be represented graphically, by points on a line A convenient method of plotting the racial points so that the frequencies not only of the genes but also of the three types can be read off directly from the same graph has been suggested by Mustakallio³³ The frequencies of the genes M and N for various populations are plotted by rectangular coordinates measuring m along the ordinate and n along the abscissa The racial points determined in this way will fall on the hypotenuse of an isosceles right triangle (cf figure 66) The distribution of M and N can also be represented by measuring the frequency of type M along the ordinate and type N along the abscissa By this method, points representing the frequencies of these types fall on a parabola The racial point on the parabola corresponding to any given point on the hypotenuse, is found by drawing a line perpendicular to the hypotenuse at the given point and determining its intersection with the parabola

Thus, with the aid of figure 66, the distribution of the properties M and N and the frequencies of the genes M and N for the populations listed in table 75 can be read off directly, and the relationship between various racial points can be determined at a glance Rather striking is the similarity of the distribution of M and N in the Blackfeet tribe³⁴ of Indians and in the other Indian tribes thus far examined in view of the differences among these tribes with regard to the distribution of the four blood groups

³² *Jour Immunol* 16: 123 (1929)

³³ *Acta Soc Med Fenn "Duodecim"* 20 No 2 (1937)

³⁴ Matson, Levine and Schrader *Proc Soc Exp Biol and Med* 33: 297 (1935), *ibid.* 35: 46 (1936)

TABLE 75
RACIAL DISTRIBUTION OF THE AGGLUTINOGENS M AND N

Nationality	Investigators	Number of Persons Tested	Per Cent of Types			σD	$\frac{m-M}{MN/2}$	$\frac{n-N}{MN/2}$
			M	N	$\frac{M+N}{\sqrt{M}+\sqrt{N}}$			
Ainu	Kuhn	504	17.86	31.94	50.20	± 2.23	43.0	57.0
Australian aborigines	McCall and Boyd	720	3.0	67.4	29.6	± 2.19	17.8	82.2
Berberians (Kwakiwilt)	Boyd and Boyd	304	56.3	7.3	36.4	± 2.18	14.5	85.5
Berberians (Kwakiwilt)	Boyd and Boyd	334	38.3	13.6	44.2	± 2.16	61.3	38.7
Belgians	Mouriau	3,100	24.87	20.77	50.36	± 0.89	54.1	45.9
Chinese	Rile	1,029	52.24	16.17	46.59	± 1.15	57.5	42.5
Danish	Harley	2,023	29.07	21.40	49.53	± 1.12	33.8	66.2
English	Harley Taylor or Thomas-Hewitt	1,522	30.48	21.56	48.16	± 1.28	54.6	45.4
French	Matla	613	23.1	23.1	48.6	± 1.3	52.6	47.4
French (East Greenland)	Labrecque-Hansen	569	63.45	0.86	15.64	± 2.1	91.3	8.7
French (St. Greenland)	Palmaus-Hansen	1,063	66.2	2.9	31.0	± 1.7	81.6	18.4
German	Recks	310	15.5	49.6	30.6	± 1.6	59.7	40.3
German (Schleswig-Holstein)	Nustallius-Streng	6,926	42.3	13.7	44.0	± 0.6	64.3	35.7
German (Schleswig-Holstein)	Nustallius-Streng	1,569	36.6	14.7	48.7	± 1.1	61.0	39.0
German (Schleswig-Holstein)	Hufschmidt, de la Riviere and Kossovitch	1,400	30.1	19.6	50.1	± 1.3	55.2	44.8
German (Schleswig-Holstein)	Schmidt	12,089	23.5	21.1	50.4	± 0.7	53.7	46.3
German (Schleswig-Holstein)	Schmidt, Blumel, Christensen, Lauenburger, Mayser, Crome, Wagner, Fuchel, Meisner, Laves, H. German	40,235	10.72	19.73	50.04	± 0.61	55.2	44.8
Hindus	Greval	300	42.7	10.7	46.7	± 2.9	76.0	24.0
Hungarians	Lenart	624	35.49	18.59	41.92	± 2.00	37.4	62.6
Indians (U.S.A.)	Landsteiner Levine	205	60.00	4.83	35.12	± 3.49	77.6	22.4
Indians (U.S.A.)	Allen, Larsen	140	59.29	7.85	32.86	± 3.85	75.7	24.3
Indians (U.S.A.)	Mason, Levine, Schrader	95	34.74	3.26	40.00	± 5.02	74.7	25.3
Irish (Dublin)	Boyd and Boyd	399	30.0	23.3	46.7	± 2.5	55.3	44.7
Italians	Latres, Grassi, Nicoletti	746	28.94	17.12	53.94	± 1.81	55.9	44.1
Japanese	Itschimoto, Hirayama, Furuhata, Imamura, Sugihara, Noguchi, Taniguchi	7,551	28.99	21.09	49.94	± 0.57	54.0	46.0
Koreans	Yamamoto	836	27.9	20.6	51.4	± 1.4	53.4	46.6
Koreans (New York)	Landsteiner Levine Wiener	328	15.52	33.84	39.64	± 2.99	53.7	46.3
Koreans (New York)	Morzyck	600	22.80	40.00	40.00	± 0.85	52.7	47.3
Koreans (New York)	Landsteiner Levine Wiener	763	32.34	21.21	46.53	± 1.81	45.5	54.5
Koreans (New York)	Landsteiner Levine Wiener	489	30.9	46.1	41.0	± 2.5	61.9	38.1
Koreans (New York)	Landsteiner Levine Wiener	456	35.09	17.10	47.81	± 2.3	69.0	31.0
Koreans (New York)	Landsteiner Levine Wiener	1,200	26.08	16.92	47.00	± 1.4	59.6	40.4
Koreans (New York)	Landsteiner Levine Wiener	110	48.1	49.6	46.3	± 2.0	41.8	58.2
Koreans (New York)	Landsteiner Levine Wiener	6,129	20.16	21.26	49.56	± 0.6	54.0	46.0
Koreans (New York)	Landsteiner Levine Wiener	192	30.7	18.0	45.3	± 1.1	41.7	58.3
Koreans (New York)	Landsteiner Levine Wiener	1,52	10.1	47.9	51.8	± 1.1	56.4	43.6

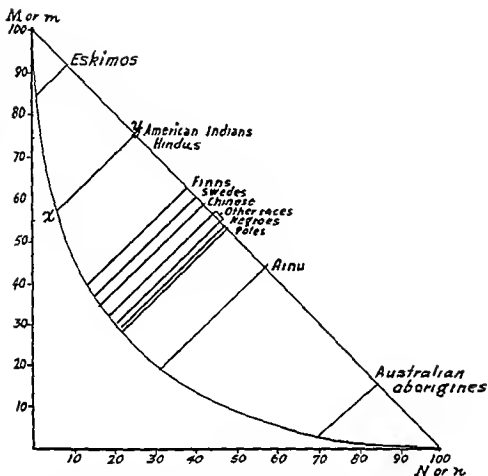


FIG 66 RACIAL DISTRIBUTION OF M AND N

Graphic representation Along one axis are measured the frequencies of type M and of gene m along the other type N and gene N (n) For example the point x gives the frequencies for American Indians of type $M=56\%$ type $N=6\%$ and point y gives the frequencies of the genes as $m=75\%$ and $n=25\%$

Kubo³² has suggested that races be classified by tests for the M and N factors with the aid of the index m/n For most races $1 < m/n < 2$ in American Indians Eskimos and Hindus $m/n > 2$ while for the Ainu and Australian aborigines $m/n < 1$

The similarity in the distribution of the M N types in the Ainu and Australian aborigines³⁴ (little M, much N) can be explained by the assumed close relationship between the origin of these peoples In contrast is the distribution of the M N factors in the Eskimos³⁷ and American Indians (much M, little N)

RACIAL DISTRIBUTION OF OTHER SEROLOGICAL PROPERTIES

While the number of investigations on other serological characters

³² Jour Immunol 30 28 (1936)

³⁴ Birdsell and Boyd Amer Jour Phys Anthropol 27 69 (1940)

³⁷ Fabricius Hansen Jour Immunol 36 523 (1939)

giving rise to individual differences in man, e.g., S, Rh and P, is still very small, the findings already collected indicate that more extensive studies may yield results of value to the anthropologist

Secretor Type The studies that have been made on this factor include only relatively small series (cf. table 76) but it appears that significant differences exist in the distribution of the property in different populations. In Europe and in the U.S.A. it has been found that about 80 per cent of white individuals are secretors and 20 per cent are non secretors³⁸. The percentage of secretors appears to be slightly higher in Finland³⁹ and markedly lower among negroes⁴⁰.

TABLE 76
STUDIES ON THE DISTRIBUTION OF THE SECRETOR TYPE

Nationality	Investigator	Number of Persons Tested	Distribution of Types		Frequencies of Genes	
			S	s	S	s
Denmark	Hartmann	100	74.0	26.0	49.0	51.0
Finland	Puthonen	197	86.3	13.7	63.0	37.0
Germany	Schiff	363	78.0	22.0	53.2	46.8
Berlin						
Japan	Suzuki	424	75.7	24.3	50.7	49.3
Japan	Isizawa Nonaka	254	79.5	20.5	54.8	45.2
Nagasaki	Okajima and Sekimata					
Poland	Morzycki	88	79.4	21.6	53.5	46.5
U.S.A. N.Y.	Schiff	74	82.4	17.6	58.0	42.0
(whites)						
U.S.A. N.Y.	Wiener	130	82.0	18.0	57.6	42.4
(whites)						
U.S.A. N.Y.	Schiff	178	61.2	38.8	37.7	62.3
(negroes)						

Agglutinin P The lack of a dependable source of reagents for testing for agglutinin P accounts for the paucity of studies on this property. Almost the only investigations on the P agglutinin have been carried out by Landsteiner and Levine⁴¹ in New York and by Dahr⁴² and his collaborators in Germany (cf. page 256). In a series of 3530 individuals Dahr found 24 per cent who were P negative and 76 per cent who were P positive and this can probably be used as a standard for European whites. Also in a short series of bloods tested in New York City the present author⁴³ has encountered about 25 per cent negative reactors.

Landsteiner and Levine found that the incidence of the P agglutinin is much

³⁸ Schiff and Sasaki *Ztschr. f. Immunitäts* 77: 129 (1932). Wiener unpublished observations.

³⁹ Puthonen *Acta Soc. Med. Fenn. Duodecim*, Ser. A14, No. 2 (1930).

⁴⁰ Schiff *Amer. Jour. Phys. Anthropol.* 27: 225 (1940).

⁴¹ *Jour. Immunol.* 16: 123 (1929) *ibid.* 18: 87 (1930).

⁴² Dahr and Zehner *Deutsch. med. Woch.* 67: 71 (1941).

⁴³ Unpublished observations.

higher in negroes than in whites in New York City Using a strong anti P immune rabbit serum these authors found 18.1 per cent negative reactors among whites and only 2.2 per cent bloods giving completely negative reactions among negroes

As has already been pointed out (cf page 260) the so-called agglutinin Q is really a variety of P agglutinin Accordingly judging from the reports on the distribution of this factor⁴⁴ one would surmise that the incidence of P negative bloods is higher in Japanese than in whites in contrast to the situation in negroes *Agglutinin Rh* As has already been mentioned (cf page 245) the Rh agglutinin was found to be present in the blood of about 85 per cent of white individuals in the U.S.A. Landsteiner and Wiener⁴⁵ found a somewhat higher incidence among negroes in New York City More recently Landsteiner Wiener and Matson⁴⁶ studied the distribution of the Rh agglutinin in American Indians and they found only a single Rh negative individual among 120 full blooded Indians examined. Results were also obtained indicating racial differences in the distribution of the subdivisions of the Rh type Levine⁴⁷ has confirmed the higher incidence of the Rh positive type among negroes and among 150 Chinese Levine and Wong encountered only a single negatively reacting blood

Other Agglutinable Properties With regard to the other agglutinogens that have been described by various investigators reagents for these properties are not available and studies on the racial distribution have been made in only one instance namely for the rare property of human blood described by Landsteiner Strutton and Chase⁴⁷ These investigators reported that this property could be demonstrated in the blood of 14 of 191 colored individuals tested but in only 2 out of 387 white individuals (cf page 264)

ADVANTAGES AND LIMITATIONS OF THE APPLICATION OF BLOOD GROUPING TESTS IN ANTHROPOLOGY

Blood tests, like other anthropological criteria, may be applied either to individuals or to groups of peoples Its application to individuals is extremely limited, however, since representatives of all four groups, all three M N types, etc., occur in almost every race, so that the tests can be used only in a few exceptional instances e.g., the occurrence of group B or subgroup A₂ would render it highly improbable that a given individual is a full blooded Polynesian or North American Indian When dealing with individuals from mixed populations, even this extremely limited application cannot be made On the other hand, certain external traits, such as skin color, type of hair, slanting eye of the Mongolian etc., tend to be more uniform in people of a common stock, and collectively usually indicate quite definitely the racial derivation of the individual The homogeneity with regard to external characters is due to intermarriage between individuals of similar racial

⁴⁴ Among 1187 Japanese Isizawa Nonaka Okayama and Sekimata [*Jap Jour Med Sci III Soc Med and Hyg* 3: 121 (1940)] found 30.7 per cent Q positive and 69.3 per cent Q negative

⁴⁵ *Jour Exp Med* 74: 309 (1941)

⁴⁶ *Jour Exp Med* 76: 73 (1942)

⁴⁷ *Science* 96: 452 (1942)

⁴⁸ *Jour Immunol* 27: 469 (1934)

type, while selection does not operate in the case of the blood group factors

Nevertheless, as has been pointed out in the preceding pages, blood grouping tests have proved to be of value for studying racial relationships and tracing racial origins. To be sure, the blood tests have limitations, because peoples of the same race may have widely different distributions, while totally unrelated races may have a similar serological constitution. However, these same limitations also apply to other anthropological criteria such as the shape of the head, stature, etc. Moreover, the blood tests have advantages over some other anthropological methods in common use. Firstly, the blood of every individual can be readily and accurately classified. Errors in technique can and have been made, but with the aid of formulae such as $p + q + r = 1$ and $m + n = 1$, reports containing such mistakes can usually be recognized. Moreover, the blood group can be diagnosed reliably at birth, even in premature infants, and it remains constant throughout life, being unaffected by environment, while such is not the case for most other anthropological criteria. Secondly, the hereditary mechanism of the individual blood properties is simple and well known, so that it is possible to predict accurately the result of any racial cross when the distribution of the blood properties in the parent populations are known. In this way it is possible to test theories concerning the racial derivation of various populations as was done in the case of the Aleuts (cf. page 320). Thirdly, the fact that there is no selection of mates on the basis of blood groups causes the blood group distribution of a population to remain constant from generation to generation in the absence of immigration and intermarriage with other races. Finally, it may be remarked that the published data regarding the racial distribution of the blood groups probably exceeds that available for any other anthropological criterion.

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CHAPTER XIX

EVOLUTION OF THE HUMAN BLOOD GROUPS INDIVIDUAL DIFFERENCES IN ANIMAL BLOOD

THE STUDY of the blood group factors A and anti A B and anti B in apes monkeys and lower animals is complicated by the presence in normal human sera of heteroagglutinins for animal blood and in animal sera of heteroagglutinins for human blood. When examining animal sera for anti A and anti B agglutinins the heteroagglutinins can be removed simply by absorption with human group O blood and the specificity of any group agglutinins remaining behind tested by absorption with group A and group B blood. However the examination of animal blood cells for agglutinogens related to the human agglutinogens A and B is more difficult. For demonstrating A and B antigens therefore Landsteiner and Miller have employed the following three techniques (1) specific absorption experiments with human sera (2) direct agglutination tests with purified solutions of human isoagglutinins (3) direct agglutination tests with anti A and anti B testing fluids prepared from immune rabbit antisera. By applying these techniques Landsteiner and Miller obtained evidence of significance for the serological relationship between man and other primates.

BLOOD GROUPS IN ANTHROPOID APES

The first grouping tests on anthropoid apes were attempted by von Dungern and Hirschfeld² who examined the blood cells of one chimpanzee. This blood gave reactions closely corresponding to group A since absorption of group B sera with human A cells removed all the agglutinins for this chimpanzee blood.

A systematic examination of blood of anthropoid apes was carried out by Landsteiner and Miller³ in 1925. These investigators established the existence in the blood of chimpanzees orang utans and gibbons of agglutinogens and isoagglutinins indistinguishable from those present in human blood. In fact despite the presence of natural heteroagglutinins in human sera for ape blood the results obtained by Landsteiner and Miller suggest that a transfusion of chimpanzee group A blood to a human being of group A would possibly cause less reaction than a

¹ *Jour Exp Med* 42 853 (1925)

² *Ztschr f Immunol* 8 541 (1911)

³ *Loc cit*

transfusion of human blood of group B Landsteiner and Miller's investigations on the blood groups in chimpanzees, orang utans and gibbons have been amply corroborated by the work of subsequent investigators⁴

Chimpanzees Of the first 14 chimpanzees examined by Landsteiner and Miller 11 proved to belong to group A and 3 to group O Subsequent tests by Landsteiner and other authors⁴ have increased the total number of chimpanzees tested to 92, of which 81 proved to belong to group A and 11 to group O⁵ To date group B and group AB have not been encountered in chimpanzees⁶

Chimpanzee blood of group A not only was agglutinated by purified solutions of human isoagglutinin α and anti A immune rabbit testing fluids but also completely absorbed the alpha agglutinins from human B sera and the sheep hemolysins from anti A immune sera so that the agglutininogen A in human and chimpanzee blood are indistinguishable⁷ Moreover strong isoagglutination reactions occurred when the serum from a group O chimpanzee was tested against group A chimpanzee bloods while sera from group A chimpanzees gave no or only faint reactions with other chimpanzee blood In absorption experiments chimpanzee A blood appears to resemble human A₁ blood more closely than A₂⁸ The sera from group A chimpanzees regularly contain β but not α agglutinins while sera from group O chimpanzees contain both agglutinins α and β in accordance with Landsteiner's rule

Orang utans The distribution of the blood groups in orang utans (*Pongo pygmaeus*) is strikingly different from the distribution in chimpanzees Among 18 orang utans tested up to 1938 7 proved to belong to group A, 8 to group B and 3 to group AB⁹ The reciprocal relationship between isoagglutinogens and isoagglutinins holds for the blood of this species Thus, isoagglutination reactions occur when bloods of group A and group B orang utans are cross matched and sera from chimpanzees of group A agglutinate blood from orang utans of group B but not of group A

⁴Landsteiner *Compt rend Soc Biol Paris* 99 628 (1928) Troisier *Ann Inst Past* 42 363 (1928), Weinert *Ztschr f Rassenphysiol* 10 7 (1938) Dahr *Ztschr f Rassenphysiol* 10 78 (1938) Wiener unpublished observations etc

⁵Cited after Dahr [*Ztschr f Rassenphysiol* 10 78 (1938)] The bloods of 5 chimpanzees not included in this total tested by Wiener and Candela (unpublished) all proved to belong to group A

⁶No attempt was made in most of these studies to distinguish the various species of chimpanzee *Pan satyrus* *Pan chimpansee* and *Pan schweinfurthii*

⁷Landsteiner and Miller *loc cit*

⁸Dahr *Ztschr f Rassenphysiol* 9 124 (1937) Wiener and Candela unpublished observations

⁹Cited after Dahr *loc cit* To these are to be added one group AB orang utan recently examined by Wiener and Candela (unpublished) If the low incidence of group AB is maintained in a larger series it would be indicative of inbreeding within separated groups of animals [Cf Haldane *Nature London* 146 652 (1940)]

Gibbons The results obtained by Landsteiner for 7 of these apes were as follows

<i>Hylobates lar</i>	1 group A, 1 group AB
<i>Hylobates leuciscus</i>	3 group B
<i>Sympholangus syndactylus</i>	2 group B

Accordingly, among these apes, just as in orang utans blood of group O has not been encountered

Gorillas The bloods of only very few gorillas were available for examination

Weinert cites the supposed occurrence of group A in every one of 4 gorillas tested up to 1938 but Dahr states that these results must be discarded for the following reasons. The blood of three of the gorillas examined by Weinert were tested with untreated human A and human B sera and these results were not valid on account of the presence in the human sera of species agglutinins for gorilla blood. The blood of the other gorilla was examined by Landsteiner who remarked that the blood of this ape could not be classified

Recently, the blood of a gorilla became available to Wiener and Candela¹⁰ The serum of the ape was found to contain strong anti A and no anti B agglutinins, but the cells gave no or only feeble reactions in anti B immune sera and did not absorb the β isoagglutinins in human A sera. This apparent lack of reciprocal relationship between agglutino-gen and agglutinin led Wiener and Candela to make further tests and extracts of the submaxillary gland were found to inhibit strongly human A sera but not human B sera.¹¹ In this particular ape therefore the expected agglutino-gen was absent or deficient in the erythrocytes, but a reciprocal relationship existed between the antigen in the glands and the agglutinins in the serum. As is pointed out below, a similar situation holds in certain species of monkeys

Two species of gorilla are recognized the lowland gorilla (*G. gorilla*) and the mountain gorilla (*G. berengei*). By tests on urine samples Candela¹² has determined the groups of 13 apes of the former species and 2 apes of the latter species. The 13 lowland gorillas all belonged to group B while both mountain gorillas belonged to group A.

BLOOD GROUP FACTORS IN MONKEYS

The regular occurrence in the sera of rhesus monkeys of anti B without anti A agglutinins, and in the sera of vervet monkeys of anti B without anti A agglutinins has been known for some time (cf. page 30). The reason for the behavior of such sera as well as sera from other monkeys was not clear since neither A nor B agglutinogens could

¹⁰ Cf. Candela, Wiener and Goss *Zoologica* 25: 513 (1940).

¹¹ Wiener, Candela and Goss *Jour. Immunol.* 45: 229 (1942).

¹² Personal communication. In a few cases organ extracts also were tested.

TABLE 77
BLOOD GROUPS FACTORS IN 16 MACAQUES

Name and Number	Family Relationship	Taxonomic Name	Reaction from Saliva	Reaction from Erythrocytes	Group Specific Agglutinin in Serum	Indicated Group
1) Rhesus	(x) Father	<i>M mulatta</i>	B	(O)*	(α)*	B
2) Rhesus	(x) Mother	<i>M mulatta</i>	B	(O)*	(α)*	B
3) Rhesus	(x) Offspring	<i>M mulatta</i>	B	(O)*	(α)*	B
4) Rhesus	(x) Offspring	<i>M mulatta</i>	B	(O)*	(α)*	B
5) Java	(y) Father	<i>M trus</i>	A	O	β	A
6) Java	(y) Offspring	<i>M trus</i>	A	O	β	A
7) Java	(y) Offspring	<i>M trus</i>	A	O	β	A
8) Java	(y) Offspring	<i>M trus</i>	A	not tested	not tested	A
9) Java	(y) Offspring	<i>M trus</i>	O	O	$\alpha \beta$	O
10) Java	(y) Offspring	<i>M trus</i>	A	not tested	not tested	A
11) Java	(y) Offspring	<i>M trus</i>	A	O	β	A
12) Java	(y) Offspring	<i>M trus</i>	A	O	β	A
13) Java	(z) Father	<i>M trus</i>	B	not tested	not tested	B
14) Java	(z) Mother	<i>M trus</i>	AB	O	none	AB
15) Java	(z) Offspring	<i>M trus</i>	AB	not tested	none	AB
16) Java	(z) Offspring	<i>M trus</i>	AB	O	none	AB

(*) These bloods were not tested because findings on the blood of rhesus have been shown to be quite constant

be demonstrated in the erythrocytes¹³ Landsteiner¹⁴ considered the behavior of the sera evidence of constitutional differences among the monkeys

After demonstrating the B property in the organs of the gorilla described above, it occurred to Wiener and Candela that the peculiar behavior of monkey blood might have a similar explanation. This received support when extracts of the submaxillary gland of rhesus monkeys were found to strongly inhibit human anti B but not anti A sera. Accordingly, a more systematic study was carried out comparing the reactions of the saliva, urine and organs of various monkey species with the isoagglutinin content of the serum¹⁵

Macaques The results of the examination of the blood and saliva of 17 macaques is presented in table 77

As is shown in table 77, the saliva of all the rhesus monkeys examined

¹³Cf Buchbinder *Jour Immunol* 25 33 (1933) Hirano Philipp *Jour Sci* 47 449 (1932) etc

¹⁴Landsteiner *Jour Immunol* 15 589 (1928)

¹⁵Candela Wiener and Goss *loc cit* Wiener Candela and Goss *Jour Immunol* 45 229 (1942)

contained the group substance B, thus accounting for the absence of the anti B agglutinins from the sera. The group reactions of the Java macaques differ from those of the rhesus, since all four groups appear to be represented in the former species. The reciprocal relationship between the reactions of the saliva and serum also holds in this species. Agglutinogens corresponding to A or B could not be demonstrated in the erythrocytes however.

Spider Monkeys Eleven individuals of two species of spider monkeys have been examined.¹⁴ No reactions occurred when the bloods of these monkeys were tested with anti A or anti B immune agglutinating sera (however cf. page 341). Judging from the reactions of the saliva and sera all six black spider monkeys examined belonged to group A. The results obtained in the case of the gray spider monkeys are more difficult to interpret since the reciprocal relationship between the reactions of saliva and serum does not appear to hold strictly for this species. One monkey gave reactions corresponding to group O but the other 4 monkeys gave results indicating the presence of B like antigens in the saliva despite the presence of β agglutinins in the serum. A probable explanation for this situation is that the fraction of the group B substance acted on by these β agglutinins is different from that present in the saliva. It is significant that the β agglutinins in the sera of gray spider monkeys were of much lower titer than the α agglutinins in the same sera and also far weaker than the β agglutinins in sera from black spider monkeys.

Conclusions More studies are necessary, but assuming that results similar to those described will be obtained in tests on additional apes and in other monkey species, certain deductions may be permissible.

Evidently, blood group substances related to A and B developed in evolution before the primates were differentiated. However in monkeys and probably in gorillas the group substances appear to be limited practically to the tissues and secretions and are absent from the erythrocytes.¹⁵ Agglutinogens so far indistinguishable from A and B are demonstrable, however, in the blood cells of chimpanzees, orang utans and gibbons so that the appearance of these group substances in the erythrocytes appears to be more recent in the evolution of man than its presence in the tissues.

As for the reciprocal relationship between group specific substances A and B and the agglutinins α and β , this holds in all the anthropoid apes and most of the monkey species examined but in gorillas and monkeys this statement applies to the group-substances in organs and secretions instead of erythrocytes. Presumably, therefore, all primates have the innate capacity to form anti A and anti B agglutinins but when substances identical or closely related to the human group-substances A and B appear in the erythrocytes and/or organs the

¹⁴ Wiener, Candela and Goss, *loc cit*.

¹⁵ This does not take into account the special B antigens present in the blood of lower animals and monkeys discussed on page 341.

formation of the corresponding agglutinins is suppressed or they are absorbed as quickly as they are produced (cf page 194) Where, as in the gray spider monkey, the group substances presumably differ qualitatively from those present in man, the reciprocal relationship appears not to hold strictly¹⁸

Of interest is the fact that some species of monkeys (e g, rhesus and vervet monkeys) and the two species of gorillas each seems to be characterized by only one of the blood group factors Moreover, in chimpanzees only groups A and O occur, while in gibbons and orang utans group O appears to be lacking This suggests that primate species with all three substances A, B, and O, may have resulted from crosses, and Landsteiner and Miller have mentioned this possibility in discussing the origin of four blood groups in man The infrequency of group O in monkeys and its absence from all anthropoids except chimpanzees is striking in view of the predominance of this group in man This suggests that gene O is more recent than genes A and B, rather than that group O was the original group as has been held by some workers (cf page 316) If gene O appeared by mutation, it could conceivably have been favored by isoimmunization in hetero specific matings (cf page 372) which might tend to eliminate offspring of a group incompatible with the mother After the appearance of the non secretor type this mechanism could conceivably have been enhanced

RELATION BETWEEN THE HUMAN ISOAGGLUTINOGENS AND ANTIGENS PRESENT IN ANIMALS (AND BACTERIA)¹⁹

As has already been mentioned, human blood A has certain reactions in common with sheep blood and these are related to that factor in sheep blood which reacts with Forssman antisera (cf page 31)²⁰ Forssman antigens, unlike the substances demonstrable by the usual precipitin reaction, which closely parallel the position of the animals in the zoological system, are scattered among animals though more or less characteristic of certain groups of related species²¹

¹⁸ Possibly because the tests were carried out with human B cells and human β agglutinins

¹⁹ For a very interesting review of this question see Friedenreich *Acta Pathol et Microbiol Scand Supp* 37 163 (1938)

²⁰ Incidentally it may be mentioned that independent of the group strong hemolytic for sheep cells are found in the serum of patients with infectious mononucleosis in which condition they are of diagnostic value and in patients after injections of horse or rabbit serum [Paul and Bunnell *Amer Jour Med Sci* 183 90 (1932) Davidsohn *Jour Immunol* 16 259 (1929) Schiff *Jour Immunol* 33 305 (1937)]

²¹ Landsteiner *The Specificity of Serological Reactions* p 55 (1936)

Forssman antigens have also been found in bacteria, such as Shiga dysentery bacilli, and certain strains of paratyphoid B bacilli and others. By immunizing rabbits with paratyphoid bacilli containing Forssman antigen, Eisler²² obtained an immune serum which hemolyzed sheep blood, and agglutinated human blood of groups A and AB, but not of group O or B. That the relationship between the antigens of the paratyphoid bacilli, sheep blood, and human blood A is not simple is evident from table 78, which summarizes some of Eisler's experiments.

TABLE 78

SEROLOGICAL RELATIONSHIP BETWEEN CERTAIN STRAINS OF PARATYPHOID B BACILLI, SHEEP BLOOD, COMMERCIAL PEPTONE, AND HUMAN BLOOD OF GROUP A (After Eisler)

Serum Treated With	Immune Serum for Paratyphoid B		Anti-Sheep Blood Immune Serum		Anti Human A Immune Serum		Normal Human Serum B α
	Hemolysis of Sheep Blood	Agglutination of Human Blood A	Hemolysis of Sheep Blood	Agglutination of Human Blood A	Hemolysis of Sheep Blood	Agglutination of Human Blood A	Agglutination of Human Blood Group A
Paratyphoid B Bacilli	-	-	+++	+++	+++	+++	+++
Sheep Blood	-	-	-	-	-	++	+
Human Blood Group A	+++	-	++	-	-	-	-
Commercial Peptone*	+++	-	+++	-	-	-	-
Saline Control	+++	+++	+++	+++	+++	+++	+++

* The reactions are attributable to the pepsin used in preparing the peptones.

Eisler²³ prepared an immune goat serum against dysentery bacilli, and found that it also agglutinated human blood. Land-teiner and Levine²⁴ examined this serum and found that it reacted most intensely with human blood of group O and subgroup A₂, and therefore resembled in its specificity the atypical human isoagglutinin, α_2 . Since, as Schiff has shown,²⁵ Shiga dysentery bacilli absorb the anti-O (anti-A₂) agglu

²² *Ztschr f Immunitäts* 73: 392 (1931)

²³ *Ztschr f Immunitäts* 67: 39 (1930), 73: 37 (1931)

²⁴ *Proc Soc Exp Biol and Med* 28: 309 (1930)

²⁵ Schiff, *Ztschr f Immunitäts* 82: 46 (1934).

tinins from Eisler's goat serum, but do not absorb the anti O (anti A₂) agglutinins present in many normal animal sera, the effect obtained with Eisler's serum was apparently caused by immunization and not due to natural agglutinins.²⁶ Recently, agglutinins acting on human blood of all groups were found to be formed on immunization of horses (but not rabbits) with pneumococci of type XIV.²⁷

The failure of certain rabbits to produce antibodies for A is due according to Witelsky²⁸ to the presence in their organs of substances related to the human A antigen on the general principle that as a rule antibodies are not formed which act

TABLE 79
IMMUNIZATION OF RABBITS WITH HUMAN A BLOOD
(After Thomsen)

Sera of Rabbits*	Titers of Unabsorbed Serum for			Titers after Absorption with		
				Human O		Human O and Sheep Cells
	Human O Agglut	Human A ₁ Agglut	Sheep Cell Hemolysins	Tested for Sheep Hemolysin	Tested for Human A ₁ Agglutinin	Tested for Human A ₁ Agglutinin
+ Group	1024	1024	64	<8	32	16
	1024	1024	128	<8	32	16
	512	512	64	<8	32	8
- Group	32	1024	2048	512	1024	512
	128	1024	4096	2048	1024	512
	512	1024	2048	1024	1024	256

* + Group—Three rabbits whose organs contain A substances

— Group—Three rabbits lacking A substances

on substances present in the body of the immunized animals.²⁹ Although exceptions have been reported³⁰ two types of rabbits can be recognized in general with respect to their response to A antigen.³¹ One group includes rabbits which lack the antigen in the tissues contain preformed anti A agglutinins in the serum and are capable of producing potent group-specific antibodies upon immunization. Rabbits of the second

²⁶ Cf Hirsfeld *Schwer Ztschr f allg Path u Bakt* 1 23 (1938)

²⁷ Finland and Curnen *Science* 87 417 (1938) Cf Haugland Beeson and Goebel *Science* 88 261 (1938)

²⁸ *Ztschr f Immunitäts* 59 139 (1928)

²⁹ Cf Treibman *Ztschr f Immunitäts* 79 274 (1933)

³⁰ Mai *Ztschr f Immunitäts* 66 213 (1930)

³¹ Dolter *Ztschr f Immunitäts* 43 92 (1925), Stuart Sawin Griffie and Wheeler *Jour Immunol* 31 31 (1936)

group have the antigen in the tissues and possess no preformed anti A agglutinins. Whereas such rabbits formerly were considered incapable of producing immune specific anti A agglutinins the formation of low titered A antibodies in such rabbits has recently been demonstrated (cf table 79).²⁷ Wheeler Sawin and Stuart²⁸ have shown that the presence or absence of preformed anti A agglutinins in the sera of normal rabbits is determined by heredity the property being transmitted as a simple Mendelian recessive. Accordingly by suitable inbreeding a strain of rabbits has been obtained by these workers which is capable of forming rather regularly high titered anti A sera which also hemolyze sheep blood.

As is the case with the human agglutinin A it has been found that a property resembling human agglutinin B is present in a large number of mammals. Thus, von Dungern and Hirschfeld²⁹ were the first to point out that the red blood cells of cattle and certain other animals can absorb human isoagglutinin β but not isoagglutinin α . Landsteiner and Miller³⁰ reached similar conclusions by making agglutination tests with purified solutions of the human isoagglutinins. These investigators found that isoagglutinin solutions prepared from group A serum gave distinct positive reactions with the bloods of rabbit, guinea pig, rat, mouse, cat, dog, steer, donkey and pig while negative reactions were obtained with bloods from sheep, horse, goat, pigeon, chicken, duck and goose. Landsteiner and Miller found that animals of the same species gave identical or practically identical reactions with the isoagglutinin solutions so that the reactions determined species rather than individual characters in animals.³¹ They also pointed out that the agglutinable substance in animals' blood is not identical with the human factor B, since anti human immune rabbit sera against human B blood does not act on the blood of animals which react with the purified isoagglutinin solutions.

As already mentioned Friedenreich and With³² have shown (cf page 292) that the human agglutinin B behaves serologically as if it had a number of components B_1, B_{11}, B_{12} all but one of which (B_1) may be present in certain animal bloods. The B agglutinogens from various animals species differ in the degree of resemblance to the human B agglutinin and according to observations made by Dahr³³ the greater or lesser similarity is not correlated with the position in the zoological scale. That the distribution of the B like agglutinogens is not entirely random however, follows from the observations of

²⁷Thomsen *Ztschr f Immunitäts* 87 335 (1936)

²⁸*Jour Immunol* 36 349 (1939)

²⁹*Ztschr f Immunitäts* 9 87 (1911)

³⁰*Loc cit*

³¹Cf Thomsen and Kemp *Ztschr f Immunitäts* 67 251 (1930)

³²*Ztschr f Immunitäts* 78 152 (1933) Cf Dupont *Arch internat de Med exp* 9 133 (1934) Fisher *Ztschr f Immunitäts* 84 136 (1935)

³³*Ztschr f Immunitäts* 91 211 (1937)

Landsteiner and Miller (cf fig 67) that B like antigens were regularly present in the erythrocytes of the New World monkeys examined (20 monkeys representing the following genera were tested *Cebus*, *Lagothrix*, *Ateles*, *Mycetes*, *Chrysothrix*, *Nyctipithecus*, also representatives from the families of marmosets and lemurs), while such antigens could not be demonstrated in the bloods of Old World monkeys (57 monkeys from the following genera were tested *Papio*, *Cercopithecus*, *Cercocebus* and *Macacus*)

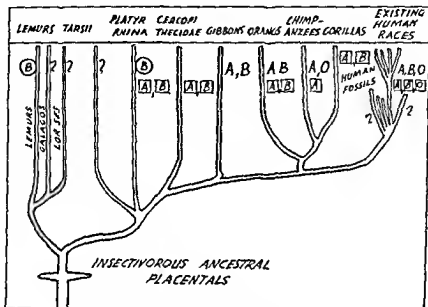


FIG 67 BLOOD GROUP FACTORS IN MAN AND IN MONKEYS
(Modified after Landsteiner and Miller)

A = agglutinin of human Group A red cells

B = agglutinin of human Group B red cells

Encircled B = agglutinin similar to but not identical with B

O = blood and serum corresponding to human Group O

Boxed A B = group substances corresponding to A and B in the tissues

? = not examined

In deer mice (genus *Peromyscus*) Cotterman²⁰ found B like antigens present in some species (*leucopus*, *maniculatus*, *polionotus*) and practically lacking in others (*eremicus*, *nasutus*, *crinitis*, *truei*). From some preliminary observations on species crosses it seems that this species difference between bloods of deer mice with regard to the B like antigens is genetically determined.

According to available data with only few exceptions the agglutinogens determining individual differences in animal erythrocytes are unrelated to the properties A and B of human blood. The so-called agglutinin A present in swine blood of

²⁰ Personal communication.

group Ao is apparently somewhat related to the human agglutinin A⁴⁰ since human A blood absorbs the anti A agglutinins present in swine sera O_x although swine blood A only partly absorbs human anti A agglutinins (cf. page 331)

Herman⁴¹ has reported certain observations which if corroborated would indicate that the properties A and B of horse blood are identical with the corresponding properties of human blood. In a review of Herman's article Schermer⁴² flatly contradicts Herman's contention and Friedenreich and Thyssen's⁴³ report supports the latter view. On the other hand substances closely resembling in serological tests the human group-specific properties A and B have been shown to occur in the saliva of certain horses in high concentration (equivalent to their concentration in human saliva)⁴⁴. Based upon the reactions given by saliva Friedenreich and Thyssen have divided horses into four groups O A B and AB similar to the human blood groups. Among 52 horses examined one belonged to group O 44 to group A 3 to group B and 4 to group AB. Substances reacting similarly to the group-specific substance A have also been found in the saliva of cattle and pigs 49 per cent of 83 cows and 50 per cent of 60 pigs were found by Friedenreich to possess the property A.

The group reactions of the salivas and sera from 21 cattle were studied for purposes of comparison with the behavior of similar material from monkeys⁴⁵. After absorption of the heteroagglutinins for human blood almost all the bovine sera were found to contain anti A agglutinins while about half had anti B agglutinins. Only a few of the bovine salivas gave distinct inhibition reactions with human A and B sera and these did not appear to be related to the reactions of the corresponding bovine sera. Moreover two sera contained anti O agglutinins as well as anti A and anti B agglutinins which could hardly occur if the organs or blood of these cattle had group-substances identical with human O A or B. Accordingly cattle saliva and sera differ strikingly from similar material from monkeys just as would be expected. It is of interest to note that Hartmann⁴⁶ found that on the average the higher the inhibition titer of bovine saliva for human B sera the lower was the titer of the anti A agglutinins in the corresponding bovine serum. In this respect there is a certain reciprocal relationship between group-substances in saliva and group-agglutinins in the sera also in cattle.

THE AGGLUTINOGENS M AND N IN MONKEY AND APE BLOOD

Apparently contradictory observations with regard to the presence of M and N agglutinogens in monkey blood have been explained by differences among the M and N agglutinins present in various anti human immune rabbit sera⁴⁷. At present, it is definitely established that

⁴⁰ Szymanowski, Stetkiewicz and Wachler *Compt rend Soc Biol* 94 204 (1926) 95 932 (1926) Hirszfeld and Halber *Ztschr f Immunitats* 59 1 (1928) Szymanowski and Frendzel *Ztschr f Immunitats* 88 379 (1936)

⁴¹ *Jour Immunol* 31 347 (1936)

⁴² *Deutsch Ztschr f d ges gerichtl Med Ref* 29 158 (1937)

⁴³ *Compt rend Soc Biol* 126 801 (1937)

⁴⁴ Brahn and Schiff *Altn Woch* 8 1523 (1929) Friedenreich and Thyssen *loc cit*

⁴⁵ Wiener and Ferguson unpublished observations

⁴⁶ Hartman *Human Like A Antigens in Cattle* Copenhagen (1941)

⁴⁷ Wiener *Jour Immunol* 34 87 (1938) Landsteiner and Wiener *Jour Immunol* 33 19 (1937) 30 per cent of 58 anti M sera tested by Wheeler and Stuart [*Jour Immunol* 37 169 (1939)] were found to act on rhesus blood

properties serologically related to the agglutinogens M and N of human blood exist in chimpanzee blood,⁴⁸ and M agglutinogens have also been found in the blood of orang utans, gibbons⁴⁹ and in all species of Old World monkeys tested.⁵⁰ Also anti M immune sera could be produced by immunizing rabbits with the blood of *M rhesus*.⁵¹ On the other hand reactions for M or N have not been obtained with the blood of most New World monkeys or of lower animals such as cattle, sheep, rabbits, guinea pigs, chickens etc.⁵²

In table 80 are given the reactions which were obtained with 6 different anti M testing fluids in tests with blood taken from a variety of monkey species. It will be seen that the bloods of some species of monkeys can be distinguished by the differences in their reactions with the various M fluids. In general, more reactions were obtained with sera of higher titers but that the differences were not merely quantitative was proved by titration and absorption tests (cf table 81). Moreover, there seems to be some correlation between the place in the zoological scale and the degree of similarity between the M substances in the blood of monkeys and that present in human M blood.

The reactions outlined in table 81 may be most simply explained by postulating the existence of at least two sorts of M agglutinins: one acting exclusively on human M blood, the other acting equally on human M and rhesus bloods. When the reactions of the various M testing fluids with various species of apes and monkeys were compared at least six qualitatively different anti M agglutinins could be differentiated.

The formation of qualitatively different anti M and anti N agglutinins on immunizing rabbits with human M and N blood respectively can be explained more reasonably by the production of more than one antibody in response to a single antigen, possibly but not necessarily due to the presence of discrete groups in the antigen molecule (Landsteiner) rather than by assuming the presence of several discrete but not Mendelizing antigens in human M and N blood (cf page 293).

It is evident that the studies on the M agglutinogens in the bloods of apes and monkeys provide further evidence of biochemical evolution.

Landsteiner and Levine *Jour Exp Med* 47: 757 (1928). Wiener *loc cit*.
Dahr *Ztschr f Immunolats* 90: 376 (1937).

⁴⁸ Wiener *loc cit*.

⁴⁹ Landsteiner and Wiener *loc cit*. Dahr *Ztschr f Rassenphysiol* 8: 145 (1936). Wheeler and Stuart *Jour Immunol* 37: 169 (1939).

⁵⁰ Landsteiner and Wiener *loc cit*. Wheeler and Stuart [*Jour Immunol* 37: 169 (1939)] have also succeeded in producing anti M sera by injecting rhesus blood into rabbits. Moreover, when rhesus monkeys were injected with human M blood they failed to produce M agglutinins though they readily formed species specific antibodies.

⁵¹ Landsteiner and Levine *loc cit*. Wiener unpublished observations. Neither could Holzer demonstrate these properties in liver or kidney tissue of lower animals [*Deutsch Ztschr f d ges gerichtl Med* 26: 518 (1936)] although Dupont [*Arch Internat de Med Exper* 9: 133 (1934)] reported such results.

TABLE 80
M AGGLUTINOGENS IN MONKEY BLOOD

Source of Blood Specimens	Anti M Testing Fluid*					
	M5	M1	M21	M35	M2	M82
Human M	+++	+++	++±	++±	+++	++±
Human N	0	0	0	0	0	0
Chimpanzee	+++	+++	+++	++	+++	±
Old World monkeys (Cercopithecidae)						
Sphinx Baboon	+++	++	+++	0		0
Drill Baboon	+++	+++	++±	(+)	(+)	(+±)
Chacma Baboon	+++	+++	++±	0	tr	0
<i>M. rhesus</i>	+++	+++	++±	(+±)	±	0
Java Macaque	+++	+++	+±	0	0	0
Sooty Mangabey	+++	+++	++±	tr	±	+
Green Monkey	+++	+++	0	0	0	0
New World monkeys (Platyrrhina)						
White Spider Monkey	++±	0	0	0	0	0
Black Spider Monkey	±	0	0	tr		0
Wooly Monkey	0	0	(±)	0	0	0
Brown Ringtail (Capuchin Monkey)	0	0	0		0	0
Moss Monkey	0	0	f tr	±		0
Lemur	0	0	0	0	0	0
Average Titer of Testing Fluids	64	64	32	24	16	16

Of several species two individuals were tested, of brown ringtails 4, of *Macacus rhesus* 45

Reactions placed in parentheses were found not to be removed by absorbing the sera with human M blood. In addition there are a number of weak reactions which were not tested with human M blood but probably belong to the same category.

* These were prepared from the immune sera in the usual way, by diluting with saline and then absorbing with packed human N cells (cf. chapter XIII).

in addition to that supplied by the investigations on the blood group factors (A and B), and on serum proteins.⁶² Thus, the M agglutinogens in ape bloods resemble the human M agglutinin more closely than the M agglutinogens in monkey bloods do (cf. table 80), and in several monkey species considered more distant from man attempts to demonstrate the presence of M agglutinogens have thus far been unsuccessful.

⁶² Nuttall *Blood Immunity and Blood Relationship* Cambridge: The University Press (1904)

TABLE 81

REACTIONS OF RHESUS AND HUMAN BLOOD WITH THREE DIFFERENT M FLUIDS

Testing Fluid	Blood of	Dilution of Testing Fluid								
		1 1	1 2	1 4	1 8	1 16	1 32	1 64	1 128	
M1, Before Absorption	Rhesus	+++	+++	++	+	+	f tr	-	-	
	Human M	+++	+++	+++	+++	++	+	+	-	
M1, Absorbed with Rhesus Blood	Rhesus	-								
	Human M	+++	+++	++	+	+	-	-		
M1 Absorbed with Human M Blood	Rhesus	-								
	Human M	-								
M5 Before Absorption	Rhesus	+++	+++	+++	++	tr	-	-		
	Human M	+++	+++	+++	+++	++	+	-		
M5, Absorbed with Rhesus Blood	Rhesus	-								
	Human M	tr	-							
M5 Absorbed with Human M Blood	Rhesus	-								
	Human M	-								
M82, Unabsorbed	Rhesus	tr	-	-						
	Human M	+++	+++	+	+	+	-			

Among anthropoid apes, the M agglutinin in chimpanzees has been found to be most like the human M agglutinin, moreover, this is the only species in which N agglutinogens have thus far been demonstrated.⁵⁴ Accordingly, these findings conform with the generally accepted view that the chimpanzee is the most man like of the anthropoid apes.

INDIVIDUAL DIFFERENCES IN ANIMAL BLOOD

Methods of Demonstrating Individual Differences in the Blood of Animals In general the methods that are applied to demonstrate individual differences in the blood of animals are the same as those used for human blood. Thus, normal or immune heteroagglutinating sera⁵⁵ may differentiate bloods of animals of the same species after the species specific agglutinins have been removed from the sera by absorption,

⁵⁴ Wiener *loc cit*, Landsteiner and Levine *loc cit*.

⁵⁵ Also heterogenetic immune sera have been successfully employed for demonstrating individual differences in fowl blood (cf page 352).

natural isoagglutinins have likewise served to demonstrate individual differences among the bloods of animals of the same species. The method of attack that has been most successful is the use of immune isoantibodies (agglutinins and hemolysins).

The first experiments along this line were made by Ehrlich and Morgenroth⁴ shortly after the discovery of the human isoagglutinins. These authors found that when one goat was injected with the bloods of other goats, immune hemolysins appeared in its blood serum. With the aid of these 150 immune sera, a number of individual varieties of goats' bloods could be distinguished. By the same method, a number of investigators have demonstrated individual differences in the bloods of dogs, cats, rabbits, cattle, sheep, chickens and monkeys.

Another method of determining cellular individuality has been described by Irwin and Hill.⁵⁷ These investigators made several parabiotic unions between backcross hybrids during a study on hybridization between Pearlneck and Ring doves. Whereas no isoagglutinins had been found previously in the Ring dove species or in the backcross hybrids, it was found that each member of a pair of parabiotic twins developed antibodies against the red cells of the other, presumably on account of reciprocal immunization.

*Isoagglutination in Lower Animals*⁵⁸ Isoagglutination occasionally of marked intensity, occurs with the blood of certain lower animals (sheep, pigs, cattle, horses). On the other hand, according to most investigators, in many animal species, such as lower monkeys, rabbits, guinea pigs, dogs, cats, mice and fish, isoagglutination reactions are either rare or entirely absent. Even in those species of lower animals in which isoagglutination reactions occur, these do not as a rule determine as sharply defined groups as in man and apes.

The reasons why bloods of lower animals do not fall into sharply defined groups are the following. Firstly, the reactions are often weak, so that the contrast between positive and negative reactions may not be striking; secondly, the reciprocal relationship between the absence of agglutinogens and the presence of agglutinins often does not hold; and finally, the variety of isoagglutinins is frequently greater than in man. Nevertheless, on the basis of the strong reactions in certain species a division into groups has been suggested, as will be discussed later on *Anthropoid Apes*. As has already been pointed out, with the isoagglutination reaction blood groups, indistinguishable from those present in man, can be demonstrated in chimpanzees, orang utans and gibbons. Isoimmunization experiments on anthropoid apes, which might per-

⁵⁷ *Berlin klin Woch* 33: 453 (1900).

⁵⁸ *Proc Soc Exp Biol and Med* 33: 566 (1936).

⁵⁹ For a thorough review of this question see Thomsen, *Handbuch der Blutgruppenkunde*, p. 88 (1932), Schäper, *Zeitschr Züchtg* 20: 419 (1931).

mut inferences on the conditions in man, have not yet been carried out. However, Landsteiner and Levine⁵⁰ injected chimpanzees with human blood, and in addition to species specific agglutinins for human blood noted the appearance in these animals of isoagglutinins that acted on certain chimpanzee bloods independently of the blood group.

Lower Monkeys No isoagglutination reactions have been reported with normal blood of lower monkeys (*M. rhesus*⁶⁰ *M. cynomolgus*⁶¹). With the aid of immune isoagglutinins, Fischer and Klinkhart⁶² succeeded in demonstrating an agglutinin in the blood of 26 out of 30 rhesus monkeys. Monkeys not possessing the factor in question formed isoagglutinins and isohemolysins against it when injected with suitable blood. On the other hand, monkeys possessing the factor did not form antibodies.

Cattle In this species, strong isoagglutination reactions have been obtained with natural isoagglutinins, but it was found difficult to make a definite classification into groups on that basis.⁶³ On the other hand the studies on cattle carried out with immune isoantibodies deserve special consideration, since it was possible in this way to differentiate the blood of practically every individual.

In 1910 Todd and White⁶⁴ studied the sera of cattle which in the course of immunization for the production of cattle plague immune serum had been inoculated with large quantities of blood (2 to 4 liters) of other cattle. These sera were found to be actively isolytic (but not autolytic), after the addition of guinea pig serum (to provide complement). A number of the more potent sera were pooled to form a mixture which had the capacity of hemolysing all cattle blood. When the pooled sera were exhausted with the blood of one ox it was possible by means of this reagent, to single out the absorbing blood from the bloods of 110 other cattle, since the absorbed sera actively hemolysed all bloods except the one used for absorption. The only exceptions found were in genetically related animals. Todd and White concluded, therefore that in cattle the blood of each individual possesses specific characteristics distinguishing it from the blood of any other unrelated individual.

One way to explain these results would be to assume the existence of a special agglutinin in each individual blood.⁶⁵ Absorption with the

⁶⁰ *Jour Immunol* 22 397 (1932)

⁶¹ Buchbinder *Jour Immunol* 25 33 (1933) Wiener unpublished observations etc

⁶² Fischer and Klinkhart *Ztschr f Immunitats* 75 513 (1932)

⁶³ Fishbein *Jour Inf Dis* 12 133 (1913) Hirsfeld and Halber *Ztschr f Immunitats* 59 17 (1928) Little *Jour Immunol* 17 377 391 (1929) etc

⁶⁴ *Proc Roy Soc B* 82 416 (1910) *Jour Hyg* 10 185 (1910)

⁶⁵ This explanation would conflict with established genetic principles

TABLE 82

ILLUSTRATING THE MECHANISM DETERMINING THE INDIVIDUALITY
OF THE BLOOD IN ANIMALS

Polyvalent serum absorbed with blood of	Agglutinins in absorbed sera	Absorbed sera tested against red blood cells of									
		1 ABC	2 ABD	3 ABE	4 ACD	5 ACE	6 ADE	7 BCD	7 BCE	9 BDE	10 CDE
1 ABC	de	-	+	+	+	+	+	+	+	+	+
2 ABD	ce	+	-	+	+	+	+	+	+	+	+
3 ABE	cd	+	+	-	+	+	+	+	+	+	+
4 ACD	bc	+	+	+	-	+	+	+	+	+	+
5 ACE	bd	+	+	+	+	-	+	+	+	+	+
6 ADE	bc	+	+	+	+	+	-	+	+	+	+
7 BCD	ac	+	+	+	+	+	+	-	+	+	+
8 BCE	ad	+	+	+	+	+	+	+		+	+
9 BDE	ac	+	+	+	+	+	+	+	+		+
10 CDE	ab	+	+	+	+	+	+	+	+	+	-

blood of any given individual would then remove only the corresponding specific agglutinin. This would entail the assumption of an immense number of specific agglutinogens and agglutinins. The effect observed by Todd and White can be explained more reasonably by assuming the existence of only a relatively small number of agglutinogens²² forming various combinations in the bloods of the different cattle.

To illustrate the probable mechanism involved let us consider a hypothetical experiment with only ten different bloods. Suppose that the polyvalent serum prepared by cross-immunizing these animals agglutinates (or hemolyzes) the bloods of all ten animals and that when the serum is absorbed by the blood of any of the animals until it no longer reacts with it, it still retains its ability to react with the other nine bloods. This effect can be explained by assuming the existence of only five agglutinogens (cf. table 82). Thus if the serum containing agglutinins a, b, c, d, e is mixed with blood No. 1 containing agglutinogens A, B, C, the agglutinins a, b, and c will be absorbed and d and e will remain unaffected. Since all the other nine bloods contain one or both of the agglutinogens D and E, whereas blood No. 1 contains neither agglutinin, the serum will react with all the bloods except No. 1. In a

²²Landsteiner and Levine *Proc. Soc. Exp. Biol. and Med.* 30: 209 (1932).

similar way the other reactions are obtained (In this hypothetical experiment, we have arbitrarily chosen bloods containing the same number of agglutinogens.) With larger numbers of agglutinogens, the number of individual differences increases rapidly. Thus, 20 different agglutinogens would give rise to as many as 200 000 differently reacting bloods. It is clear, therefore, that the assumption of a special agglutinin for each blood to explain the results obtained by Todd and White, is unnecessary.

In a series of fundamental investigations, Ferguson⁶⁶ and Ferguson, Stormont and Irwin⁶⁷ have identified a large number of unit factors in cattle erythrocytes determining individual differences in the blood of this species. From isoimmune sera and immune rabbit sera, these workers have prepared thirty reagents, each specific for a different, presumably unit antigen. They point out that if one assumes the thirty agglutinogens to be independent of each other, then their reagents would make possible classification of cattle into 2^{30} or over a billion distinct blood types. Moreover, they remark that there is evidence for the existence of additional blood factors which would increase "the number of possible combinations to a staggering total not even approached by the national debt at the present writing, i.e., beyond human comprehension." It is not surprising, therefore, that in tests on hundreds of cattle with their 30 reagents, Ferguson, Stormont and Irwin have not yet found two animals, even among blood relatives (excluding identical twins) which gave completely identical reactions.

In heredity studies, every one of the 30 agglutinogens proved to be inherited as a simple mendelian dominant. In a large series of matings (150 matings for the most recently discovered antigens, and more than 350 matings for those first detected) not a single animal was encountered which possessed an agglutinin lacking from the blood of both its parents. These heredity results furnish further evidence of the unit character of each of the 30 agglutinogens and at the same time demonstrate that none of them resulted from the complementary action of genes, such as have been shown to occur in species crosses in pigeons by Irwin (cf. page 357), and within the species in chickens by Thomsen (cf. page 354). Some of the agglutinogens have been found to have a serological relationship somewhat analogous, for example, to that existing between the properties A_1 and A_2 of human blood, which would tend to exclude the possibility that the antigens are all inherited independently. Obviously the 30 antigens can be put to very practical use in the cases of disputed parentage that sometimes arise in connection with the registration of cattle.

Many of the antisera were produced by weekly transfusions of a liter of blood from a cow to her dam, close blood relatives being selected in order to reduce the number of antigenic differences between donor and recipient. In a few instances the immunized animal developed potent type specific isoantibodies not absorbable by, and therefore presumably lacking from the donor's cells. These, Ferguson, Stormont and Irwin have attributed to the non specific stimulating action of the transfusions. Sheep Isoagglutination and isohemolysis of varying intensity and specificity have

⁶⁶ *Jour. Immunol.* 40: 213 (1941)

⁶⁷ *Jour. Immunol.* 44: 147 (1942).

— *Journal of Animal Biology* 14: 1 (1943)

been observed with the blood of normal sheep⁶⁸ If only the stronger and more consistent reactions are considered sheep blood can be divided into three groups Ro O anti R and Oo That the agglutinin R of sheep blood is related to though not identical with the agglutinin A of human blood follows from the fact that human A blood absorbs the agglutinin anti R of sheep serum but sheep blood R only partially absorbs the human isoagglutinin anti A (cf page 342) Moreover Andersen⁶⁹ has shown that the so-called agglutinin R is not a single property but includes a group of serologically related substances analogous to the agglutinogens A₁ A₂ of human blood Kaczkowsky⁷⁰ examined the bloods of 214 lambs and their parents As is shown in table 83 Ro is dominant over Oo and O anti R and O anti R is dominant (or epistatic) to Oo

TABLE 83
HEREDITY OF THE BLOOD GROUPS IN SHEEP
(After Kaczkowsky)

Parental* Combination	Groups of Offspring			Number of Offspring
	Ao	Oo	Oα	
Ao × Ao	17	2	—	19
Ao × Oα	13	1	—	14
Ao × Oo	49	—	1	50
Oα × Oα	—	1	6	7
Oα × Oo	—	—	19	19
Oo × Oo	—	105	—	105
Totals	79	109	26	214

* λ and α in this table correspond to R and anti R in the text

Todd and White¹ were the first to demonstrate individual differences in sheep blood with the aid of immune isohemolysins. Andersen succeeded in obtaining group-specific antibodies by immunizing rabbits with sheep blood and he found that these antibodies as well as the immune isoagglutinins were qualitatively different from the natural isoantibodies since the former defined individual differences independent of the three groups Ro O anti R and Oo and were not absorbable by human A blood

As has been previously pointed out (cf page 339) certain rabbits on injection with human A blood produce potent sheep hemolysins² By suitable absorption tests Andersen² was able to demonstrate that the sheep cell lysins in different anti A immune rabbit sera are qualitatively different and that such sera could be used for demonstrating individual differences in sheep blood Thus one serum was shown to contain at least 5 qualitatively different sheep lysins The reactions given by the sheep cell lysins in the anti A immune sera were found to be somewhat correlated with the groups Ro O anti R and Oo

⁶⁸ For a review of literature see Andersen *Ztschr f Rassenphysiol* 7 171 (1935)

⁶⁹ *Ztschr f Rassenphysiol* 10 88 (1938)

⁷⁰ *Compt rend Soc Biol Paris* 93 386 (1928) *Proc Roy Soc Edin* 48 10 (1927)

¹ *Loc cit*

² *Ztschr f Rassenphysiol* 10 104 (1938)

Horses As a rule isoagglutination reactions in horses are considerably weaker than isoagglutination in man but in tests on 42 horses Hirschfeld and Przesmycki¹² were able to distinguish four blood groups analogous to the groups in humans¹⁴

While most writers agree that horses can be classified in four groups on the basis of the isoagglutination reactions many investigators¹⁵ have found that a certain proportion of horses (5 to 20 per cent) do not fit into such a classification. This apparent discrepancy has been explained by Kaempffer⁶ who described the occurrence of 6 pairs of agglutinogens and agglutinins in horse blood designated by him as A α B β C γ D δ E ϵ and F ζ respectively. A reciprocal relationship between the absence of agglutinogens and the presence of agglutinins (analogous to Landsteiner's law of the blood groups in man) holds only for the first two factors whereas the other isoagglutinins though they are specific and occasionally of rather high titer are present in only a very small percentage of horses lacking the corresponding agglutinin. This accounts for the apparent division into only four groups and at the same time for the variable number of exceptions to the four group scheme.

Kaempffer has studied the heredity of the agglutinogens A B C D E and F in 260 families of horses with 361 offspring and finds that these properties are transmitted as Mendelian dominants. On the basis of his findings Kaempffer believes that blood grouping can be applied for determining non paternity in horses which may prove of practical value in checking the parentage of pedigreed horses. Kaempffer found that in 128 artificially constructed cases of doubtful parentage nonpaternity could be established 39 times and in practice 2 out of 7 cases were solved.

Lehnert¹⁷ has made an elaborate study of the individual blood differences in horses with the aid of isoimmune sera. He remarks that such sera gave more satisfactory results than natural isoagglutinating sera because of their higher titer. The tests were carried out by the technique of isohemolysis (adding guinea pig complement) and isoagglutination. Lehnert found that isoimmune sera were most readily obtained for two major blood factors designated by him as A and B the situation in this regard being somewhat analogous to that existing in man. These two factors determined four major blood groups but this situation is complicated by the existence of variations in the A and B receptors. For example in his so called group A Lehnert claims to have identified a main antigen and 5 partial receptors while in category B he asserts there are at least 3 sorts of receptors. In addition Lehnert described three so called lysinogens and various minor isoagglutinogens in horse blood. When intergroup transfusions (e.g. A to B) were given severe and even fatal hemolytic reactions were observed not only in horses receiving repeated injections but also occasionally at the very first transfusion and Lehnert ascribes the latter to the presence in the recipients of natural isoantibodies against A and B. For further details the original monograph should be consulted.

Pigs Knowledge concerning individual blood differences in pigs is limited at the

¹² *Compt rend Soc Biol Paris* 89 1360 (1923)

¹⁴ Similar results were obtained by Newdow [*Zentralbl Bakteriol Ref* 90 157 (1928)] in tests on 1730 horses and by Thomoff [*Arch Tierheilk* 1 433 (1930)] in tests on 100 horses.

¹⁵ Schermer *Ztschr f Immunitäts* 58 130 (1928) Schermer and Hoffert *Ztschr f Immunitäts* 67 497 (1930) Herman *Jour Immunol* 31 347 (1936), etc.

¹⁶ *Deutsch Ztschr f d ges gerichtl Med* 25 231 (1935)

¹⁷ Lehnert *Ein Beitrag zur Kenntnis der Bluttypen des Pferdes mit Hilfe art eigenen, hochwertiger, gruppenspezifischer Isoimmunsera*, 336 pages Uppsala (1939)

present time to the results of studies on isoagglutination. On the basis of the isoagglutination reactions pigs (like sheep) have been divided into three groups Ao Oz and Oo. According to the literature this subdivision of pigs would seem to be supported by tests made on a total of more than 2000 animals.⁸ However, some recent studies on isoagglutination in pigs by Hardt⁹ indicate that the situation is probably far more complicated. From his investigations it seems that there are several different agglutinable properties in pig erythrocytes which can be detected by normal isoagglutination. This author confirms the existence of bloods which contain neither agglutinogens nor agglutinins from which it follows that there is no reciprocal relationship between agglutinogens and agglutinins in pig blood such as holds for the human blood groups.

Kaempffer¹⁰ has studied the heredity of blood groups in pigs. Since in his studies only three groups Oo Ao and Oz were taken into account his work may have to be re-interpreted in the light of the findings reported by Hardt.

Fowl. Whereas distinct isoagglutination reactions are only relatively rarely obtained with normal fowl blood,¹¹ individual differences in such blood can readily be demonstrated by other methods. Thus Landsteiner and Miller found that a large number of distinct agglutinogens could be demonstrated in the bloods of chickens (and ducks), by means of suitably diluted and absorbed anti fowl immune rabbit sera. It is of interest to note that Landsteiner and Levine¹² have found that one particular type of fowl blood could readily be demonstrated with the aid of normal bovine sera whereas a second distinct type of blood was defined by Forssman immune sera.

As has been shown by Todd,¹³ however, still more striking results may be obtained by using isoimmune agglutinating sera. In a manner similar to that described for cattle, the blood of any particular chicken can be differentiated from the bloods of other chickens.

Todd also obtained evidence that the agglutinable substances determining the individuality of chicken blood are inherited as Mendelian dominants just like the known human agglutinogens. Thus in three families of chickens he found that if the polyvalent serum was absorbed with the bloods of both parents it lost the power to agglutinate the bloods of the offspring though retaining the power to agglutinate the bloods of unrelated chickens. This rule was found to hold with only one exception among the offspring of the three families and this exception could

⁸ Szymanowsky, Stetkiewicz and Wachler *C r Soc de Biol* 94 204 (1926) Dohrmann *Diss Budapest* 1930 ref *Zuchtungskunde* 5 115 (1931) Kaempffer *Ztschr f indukt Abstamm u Vererbung* 61 261 (1932) etc.

⁹ *Ztschr f Rassenphysiol* 9 178 (1937) Also see Jettmar *Ztschr f Immunol* 65 288 (1930) Fishbein *Jour Inf Diseases* 12 133 (1913).

¹⁰ *Loc cit*

¹¹ Landsteiner and Miller *Proc Soc Exp Biol and Med* 22 100 (1924) Karshner *Jour Lab and Clin Med* 14 346 (1928) Shimizu *Tohoku Jour Exp Med* 18 97 (1931).

¹² *Proc Soc Exp Biol and Med* 30 209 (1932).

¹³ *Proc of the Royal Soc B* 106 20 (1930), 107 197 (1930).

TABLE 84

MEMBERS OF TODD'S "WHITE" FAMILY OF CHICKENS GROUPED ACCORDING TO SIMILARITY OF THEIR REACTIONS

Immune Serum Exhausted with Corpuscles of	Tested on Corpuscles of																C = complete agglutination ++ = very marked agglutination + = trace of agglutination			
	Γ	26	40	53	M	24	39	41	20	22	36	52	69	34	68	23		27	38	19
Father	-	-	-	-	-	ac	C	C	C	C	C	C	C	C	C	-	-	+	+	+
Chick No 26	-	-	-	-	-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Chick No 40	-	-	-	-	-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Chick No 53	-	-	-	-	-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Mother	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 24	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 39	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 41	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 20	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 22	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 36	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 52	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 69	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 34	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 68	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 23	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 27	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 38	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 19	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+
Chick No 248	C	C	C	C	C	-	+	+	+	+	-	ac	+	+	+	+	+	+	+	+

C = complete agglutination

+ + + = very marked agglutination

+ = trace of agglutination

be explained satisfactorily by the fact that the chick involved had been assigned to the wrong family

In an investigation by Thomsen⁴⁴ the heredity of individual differences in chickens was studied in 33 families with 95 offspring. Confirming Todd's results, Thomsen found that when his polyvalent isoimmune serum was exhausted with the bloods of both parents it no longer reacted with the bloods of the offspring except in three cases. Thomsen explains these three exceptions by assuming the existence of agglutinogens which are inherited as recessives.⁴⁵ These findings of Thomsen may be compared to the common case in humans of children of group O with parents both belonging to subgroup A₁. In such cases tests with an anti O (α_2) serum would give positive reactions with the children's bloods but no reaction with the parents' bloods.

By analyzing Todd's results it is possible to construct a hypothesis estimating the number of different agglutinogens present in fowl blood and the mechanism of their heredity. The polyvalent serum used by Todd was absorbed with the red blood cells of each member of each family until it no longer reacted with the absorbing blood. The absorbed sera thus prepared were then tested against the bloods of every other member of the family. The results as originally presented appeared very complicated but as the present author has pointed out⁴⁶ many of the bloods are similar and can be grouped together. As an example the reactions in Todd's white family are given in table 84. To be sure there appear to be a small percentage of inconsistencies but when the massive nature of the investigation is realized especially since the tests did not allow of repetition the results are rather satisfactorily consistent. Indeed two bloods ought to be identical if each absorbs all the agglutinins for the other like the pairs mother and chick 24 and chicks 36 and 69.

As may be seen in tables 84 and 85 the 20 chickens of the white family fall into 8 different types. A hypothesis which satisfactorily explains the results obtained is given in the latter table. The existence of 4 different agglutinogens A, B, C and D is postulated. According to the hypothesis the agglutinogens A, B and C are inherited by means of three corresponding allelic genes $1/B$ and C . Agglutinin D is inherited independently of the other 3 agglutinogens by means of a single pair of genes D and d . The father belonging to type ACD (genotype $ACDd$) produces 4 different kinds of sperm in equal numbers namely AD , Ad , CD and Cd . Similarly the mother produces four different kinds of ova BD , Bd , CD and Cd . The types of offspring possible and the proportion expected of each type are now readily determined as shown in table 85. The agreement between expectation and observation is satisfactory.

Similar reasoning can be applied to the other two families of chickens studied by Todd. These results indicate that despite the high degree of individual specificity revealed by Todd's polyvalent serum the presence of only a relatively small number of different agglutinogens need be assumed. Further evidence that the number of agglutinins and their corresponding agglutinogens is not very high was derived from experiments in which chickens were inbred by brother-sister matings.⁴⁷ In this way a family of 18 individuals was finally obtained each of whose blood gave identical

⁴⁴ *Hereditas* 19: 243 (1934).

⁴⁵ Similar observations have been reported by Boyd and Alley [*Jour. Heredity* 31: 135 (1933)].

⁴⁶ *Wiener Ztschr. f. indukt. Abstamm. u. Vererbungslehre* 66: 31 (1933); *Jour. Genetics* 29: 1 (1934).

⁴⁷ Todd *Proc. Roy. Soc. B* 117: 308 (1935). Also see Kozelka *Jour. Immunol.* 24: 519 (1933).

TABLE 85
REACTIONS IN TODD'S WHITE FAMILY OF CHICKENS, ILLUSTRATING MECHANISM OF HEREDITY OF AGGLUTININOGENS IN FOWL

Immune Serum Exhausted with Corpuscles of	Tested on Corpuscles								Number Offspring Observed of Each Type	Probable Agglutino- gen Content of Corpuscles	Proportion Offspring Expected of Each Type
	I	II	III	IV	V	VI	VII	VIII			
I (Father, Chicks 26, 40, 53)	-	+	+	+	+	+	+	+	3	ACD	3/16
II (Mother, Chicks 24, 39, 41)	+	-	+	-	+	+	-	-	3	BCD	3/16
III (Chicks 20, 22, 36, 52, 60)	+	+	-	+	+	-	+	+	5	ABD	3/16
IV (Chicks 34, 68)	+	+	+	-	+	+	+	-	2	BC	1/16
V (Chick 23)	+	+	+	+	-	+	+	-	1	AC	1/16
VI (Chicks 27, 38)	+	+	+	+	+	-	+	+	2	AB	1/16
VII (Chick 19)	+	+	+	+	+	+	-	-	1	CD	3/16
VIII (Chick 248)	+	+	+	+	+	+	+	-	1	C	1/16

reactions both in direct tests and in absorption tests with Todd's polyvalent iso immune sera.

In conclusion it should be pointed out that the heredity of agglutinogens in chickens by means of multiple allelic genes is of particular interest because of the analogy to the occurrence of multiple allelic blood group genes in man

Pigeons and Doves No reports have come to the author's attention which deal directly with individual blood differences in pure species of pigeons or doves. In hybrids, however, in a carefully planned and brilliantly executed series of experiments Irwin and his collaborators²⁴ have analysed individual blood differences.

As an example, let us consider the studies made on crosses between the domesticated Ring dove and an Asiatic species called Pearlneck. Immune sera prepared by injecting rabbits with blood of either species agglutinated with practically equal intensity not only the blood used for immunization, but also blood from the other species and from the F_1 hybrids resulting from crosses between the two species. Such sera when absorbed with blood of the heterologous species until it no longer reacted with the absorbing blood, still agglutinated in high dilutions the homologous blood and also the F_1 hybrid at about the same titer. Moreover, practically all of the antibodies could be removed by absorbing with blood of the F_1 hybrid.

These results could be explained by assuming the presence in each species of dove of a number of antigens in the red cells, some of which are shared by both species, others present in the cells of one but not the other. Practically all of these agglutinogens being transmitted as Mendelian dominants and being present in the homozygous form in the parent species, these would accordingly also occur in the hybrid. In backcrosses of the F_1 hybrids with the Ring dove species agglutinogens occurring only in Pearlneck doves segregated and by analysing the reactions of bloods from quarter Pearlnecks and one eighth Pearlnecks, Irwin found that there were at least 10 discrete agglutinogens characteristic of the Pearlneck species and not occurring in the Ring doves. In a similar way, by backcrossing F_1 hybrids to the Pearlneck doves, it could be shown that approximately the same number of agglutinogens were present in the Ring doves but absent from the Pearlnecks. In the back-crosses the independent segregation of the genes determining these agglutinogens gave rise to multiple individual blood differences. Taking only the agglutinogens present in the Pearl

²⁴ Irwin and Cole *Jour Exp Zool* 73: 85-309 (1936). Irwin, Cole and Gordon *Jour Exp Zool* 73: 285 (1936). Irwin and Cole *Jour Immunol* 33: 355 (1937). *idem* *Genetics* 25: 326 (1940). Irwin *Jour Genet* 35: 351 (1938). *idem* *Genetics* 24: 709 (1939). Cf. experiments on mules by Landsteiner and van der Scheer [*Jour Immunol* 9: 213-221 (1924)].

neck but absent from the Ring doves, 2nd different sorts of blood would be expected theoretically

It may be added that Irwin noted in the F₂ hybrid blood 2 possibly more agglutinogens not present in either of the parent species. To account for the appearance of these new agglutinogens one could postulate that each is determined by 2 epistatic genes only one of which is found in either parent species and in homozygous form

More recently Cumley and Irwin²⁰ have reported the successful differentiation of

TABLE 86
HEREDITY OF THE BLOOD GROUPS OF RABBITS
(After Castle and Keeler)

Mating	Number of Offspring of Group				Totals
	O	H ₁	H ₂	H ₁ H ₂	
1 O × O	7	0	0	0	7
2 O × H ₂ (het)*	9	0	10	0	19
3 O × H ₁	0	0	15	0	15
4 O × H ₁ (het)	12	9	0	0	21
5 O × H ₁ H ₂	(4)	140	162	(2)	308
6 H ₂ (het) × H ₁ H ₂	(2)	45	67	40	154
7 H ₂ × H ₁ H ₂	0	0	51	52	103
8 H ₂ (het) × H ₁ (het)	20	13	15	17	65
9 H ₂ × H ₁	0	0	0	12	12
10 H ₂ × H ₁ (het)	0	0	27	35	62
11 H ₁ × H ₁	0	8	0	0	8
12 H ₁ (het) × H ₁ H ₂	0	4	2	8	14
Totals	54	219	349	166	788

* Heterozygous

sera from Pearlneck and Ring doves with the aid of suitably absorbed precipitating immune rabbit sera. Moreover tests with selected anti Pearlneck sera absorbed with Ring dove serum detected individual differences in serum from pigeons obtained by backcrossing F₁ hybrids with Ring doves presumably due to segregation of genetically determined serum components derived from Pearlneck.²⁰

Rabbits Isoagglutination reactions have not been obtained with the blood of normal rabbits.²¹ However, with the aid of immune iso antibodies²² individual differences can be demonstrated in such blood

²⁰ *Proc Soc Exp Biol and Med* 44 353 (1940)

²¹ Cumley and Irwin *Genetics* 27 177 (1942), Irwin and Cumley *Genetics* 27 228 (1942)

²² Snyder *Jour Immunol* 9 45 (1924) Fleischer *Ztschr f Immunitats* 49 121 (1926) Weszczky *Biochem Ztschr* 107 159 (1920), etc

²³ Levine and Landsteiner *Jour Immunol* 17 559 (1929) *ibid* 21 513 (1931), Fischer and Kunkhart *Arb Staatsinst exp Ther Frankfurt* 23 63 (1930), Marcus *sen Ztschr f Immunitats* 89 1 (1936) etc

In this way, as many as five distinct agglutinogens have been identified²³

Sera against two of the five known agglutinogens of rabbit blood are obtained with relative ease and the four blood groups determined by these two agglutinogens (variously designated as A and B H_1 and H_2 and K_1 and K_2 respectively) have been studied rather extensively. Castle and Keeler²⁴ have studied the heredity of the four blood groups in rabbits (cf table 86) and their findings as well as the frequency distribution of the groups²⁵ can best be explained by postulating the existence of three allelic genes H_1 , H_2 , and O the recessive gene O being of infrequent occurrence in the random rabbit populations thus far examined. As shown in table 86 only 8 apparent exceptions to this theory were encountered among 188 rabbits and as Castle and Keeler suggest they were probably caused by technical errors which may well be expected considering the difficulties in typing rabbit blood.²⁶ On the whole there is a remarkable analogy between the heredity of the blood groups in rabbits and in man.

Castle and Keeler² have also searched for a possible genetic relationship between the four blood groups and various mutant genes in rabbits. Tests for linkage between the blood groups and five distinct pairs of genes lying in five different chromosomes all yielded negative results.

Mice As has already been mentioned isoagglutination reactions do not occur with mouse blood.²⁷ However, Gorer²⁸ has succeeded in demonstrating the existence of three different agglutinogens in mouse erythrocytes with immune rabbit sera for mouse blood.

The considerable technical obstacle offered by the small size of this animal was overcome by the following cleverly planned experiment. By using three different strains of mice which had been brother-sister inbred for more than twenty generations Gorer was able to deal with stocks practically homogeneous genetically. The pooled blood taken from mice of the same strain could therefore be treated like blood from a single individual. Thus Gorer was able to obtain sufficient blood of each variety for the required immunizations and absorptions. Although normal rabbit sera were found to react with mouse blood they did not differentiate between the three different mouse lines. On the other hand using suitably diluted and absorbed

²³ Fischer *Ztschr f Immunol* 86 97 (1935)

²⁴ *Proc Nat Acad Sci* 19 92 (1933)

²⁵ Very few rabbits of group O have been encountered among random series of rabbits thus far examined.

²⁶ Castle and Keeler believe this explanation of their exceptions more plausible than the assumption of non-disjunction or heredity by means of 2 closely linked pairs of genes (cf page 185)

²⁷ *Proc Nat Acad Sci* 19 98 (1933) In a recent study on the heredity of the agglutinogens H_1 and H_2 in rabbits on a total of 1145 offspring Knopfmacher [*Jour Immunol* 44 121 (1942)] observed excellent agreement with the theory of three allelic genes.

²⁸ MacDowell and Hubbard *Proc Soc Exper Biol and Med* 20 93 (1922) Nor did Boyd and Walker [*Jour Immunol* 26 435 (1934)] succeed in demonstrating individual differences in mouse blood with immune isoagglutinins.

²⁹ *Brit Jour Exp Path* 17 42 (1936) *ibid* 18 31 (1937)

immune rabbit sera blood of mice from any strain could be differentiated from blood of mice from either of the other two strains

In addition to the three agglutinogens demonstrable with immune rabbit sera a fourth individual property can be demonstrated in mouse blood by making tests with certain normal human sera ¹⁰⁰

Gorer has found that all four agglutinable properties of mouse blood are inherited as simple Mendelian dominants

Rats Most investigators have been unable to demonstrate isoagglutination in this species and among 2000 cross tests with bloods from tame rats Friedberger and Taslokova¹⁰¹ observed agglutination in only 2 instances However these investigators found isoagglutinins frequently in the sera of wild rats The isoagglutinins were of four sorts and determined four corresponding agglutinogens in rat corpuscles

By injecting rats resistant to Jensen rat sarcoma with the tumor or with the organs or erythrocytes of susceptible rats Lumsden¹⁰² induced the formation of immune isohemagglutinins which defined a type of rat blood

With the aid of natural and immune isoagglutinins Burhoe¹⁰³ has identified two agglutinogens in rat blood which are inherited independently of one another and determine four types of rat blood The relationship of these agglutinogens to the one described by Lumsden was not determined

Dogs While distinct isoagglutination does not occur with normal dog blood ¹⁰⁴ using iso immune sera, von Dungern and Hirszfild,¹⁰⁵ and Brokman,¹⁰⁶ were able to establish the existence of two agglutinogens in dog blood, giving rise to four blood groups The existence of individual differences in dog blood has been confirmed in the course of plasma pheresis experiments ¹⁰⁷

Individual Differences in the Blood of Some Other Animals No or only indistinct isoagglutination reactions have been obtained with the blood of cats,¹⁰⁸ guinea pigs,¹⁰⁹ cod fish ¹¹⁰ frogs ¹¹¹ and snakes ¹¹² An attempt to demonstrate individual blood differences in guinea pigs with the aid of immune isoantibodies and normal heteroagglutinins was un-

¹⁰⁰ *Jour Genetics* 32 17 (1936)

¹⁰¹ *Ztschr f Immunstats* 59 271 (1928)

¹⁰² *Amer Jour of Cancer* 32 395 (1938)

¹⁰³ Personal communication

¹⁰⁴ von Dungern and Hirszfild *Ztschr f Immunstats* 4 531 (1910) Ottenberg Friedman and Kaliski *Trans NY Path Soc* 31 49 (1911) etc

¹⁰⁵ *Loc cit*

¹⁰⁶ *Ztschr f Immunstats* 9 87 (1911)

¹⁰⁷ Wright *Proc Soc Exp Biol and Med* 34 440 (1936) Melnick Burack and Cogwell *Proc Soc Exp Biol and Med* 33 616 (1936) *ibid* 36 697 (1937)

¹⁰⁸ Ingebrigsten *Jour Exp Med* 16 169 (1912) *Munch med Woch* p 1475 (1912)

¹⁰⁹ Hektoen *Jour Inf Dis* 4 287 (1907) Weszecky *Biochem Ztschr* 107 159 (1920) Fleischer *Ztschr f Immunstats* 49 121 (1926) etc

¹¹⁰ Jensen *Ztschr f Rassenphysiol* 9 22 (1937)

Fishbein *Jour Inf Dis* 12 133 (1913)

¹¹¹ do Amaral and Klobusitzky *Ztschr f Immunstats* 77 315 (1932) Bond *Jour Immunol* 36 1 (1939)

successful¹¹³ In experiments made by Ottenberg and Thalhimer,¹¹⁴ immune isoantibodies were formed after repeated transfusions in cats so that further transfusion from the same donor resulted in severe or even fatal hemolytic reactions

RELATIONSHIP BETWEEN INDIVIDUAL AND SPECIES SPECIFIC PROPERTIES OF BLOOD CELLS

The species specific properties of erythrocytes are apparently due in part to protein constituents of the cells, but there are other species specific reactions e g, those related to the Forssman antigen, that are similar in their serological and chemical behavior to the individuality reactions (Landsteiner and van der Scheer¹¹⁵) The close relationship between species specific antigens and those determining individual differences is seen from the fact that reactions which determine individual blood differences in one species may be obtained with the blood of all members of a different species¹¹⁶ For example, the reaction which the agglutinin A has in common with sheep blood (cf page 31) is not obtained with human blood of group B or group O, in sheep on the other hand, it is shared by all members of the species Other examples are the presence of antigens B₁, B₂ in the blood of all rabbits cattle cats, etc,¹¹⁷ whereas in man these properties are restricted to individuals of groups B and AB, and the observation that in Rhesus monkeys which have been found to possess M agglutinogens, the blood of all members of the species gave identical reactions¹¹⁸

In this regard, observations made by Irwin¹¹⁹ on the blood of pigeons and doves are of significance This investigator found that each species studied was apparently homozygous for a number of species specific agglutinogens, which in the second hybrid generation gave rise to individual differences These findings confirm the impression that there is no sharp line of demarcation between the serological species and individual reactions of erythrocytes They also may have some bearing on the question how individual differences arose in man and animals¹²⁰

¹¹³ Boyd and Walker *Jour Immunol* 26 435 (1934) Wiener unpublished experiments

¹¹⁴ *Jour Med Res* 33 213 (1915)

¹¹⁵ *Jour Exp Med* 42 123 (1925)

¹¹⁶ Cf Landsteiner *Specificity of Serological Reactions* Charles C Thomas Springfield Ill (1936)

¹¹⁷ von Dungern and Hirschfeld *Ztschr f Immunol* 9 87 (1911) Thomsen and Kemp *Ztschr f Immunol* 67 251 (1930)

¹¹⁸ Landsteiner and Wiener *loc cit*

¹¹⁹ Irwin and Cole *Jour Exp Zool* 73 85 309 (1936) Irwin Cole and Gordon *Jour Exp Zool* 73 285 (1936) Irwin and Cole *Jour Immunol* 33 355 (1937)

¹²⁰ Cf Landsteiner and Miller *Jour Exp Med* 42 862 (1925) For a discussion of the nature of interspecific differences in general see Haldane *Evolution Essays* presented to E S Goodrich (1938)

CHAPTER XX

BLOOD GROUPS AND DISEASE TISSUE TRANSPLANTATION RH FACTOR IN ERYTHROBLASTOSIS FETALIS

Blood Groups and Grafts By far the most important application of blood grouping in clinical medicine is for the selection of donors for blood transfusion. This subject has been presented in detail in several of the preceding chapters.

A number of writers have stated that blood transfusion is really a form of tissue transplantation, so that blood transfusion could well be termed "blood grafting." From this viewpoint, it has been suggested that blood grouping should also be of value in selecting donors for tissue grafting, and particularly for skin grafting.

Our knowledge of transplantation is based largely on experiments performed upon animals (Loeb¹ Mann²), in which attempts have been made to transplant pieces of tissue, or even entire organs, such as the thyroid gland, kidney, heart, etc. It has been found that autotransplantation is generally successful, whereas heterotransplantation (grafting of tissue from one animal into another animal of a different species) does not succeed except in low animals. Homotransplantation (grafting between animals of the same species) also fails, as a rule, although the grafts survive for a longer period than heterotransplants. Homotransplantation is occasionally successful, however, particularly when performed between close blood relatives.³

A number of investigators have suggested that these results may be correlated with the known serological differences of the blood.⁴ There is no doubt that the failure of heterotransplantation is dependent upon biochemical species specificity, and it is very likely that the results of homotransplantation may have a similar basis. In this connection, Todd's⁵ observations on cattle and fowl are pertinent, in which he found that blood of any individual could be differentiated from the bloods of

¹ *Physiol Rev* 10 547 616 (1930)

² *Libman's Anniv Volumes*, Internat Press New York 2 757 (1932)

³ Schöne *Die heteroplastische und homoplastische Transplantation* Berlin (1912), Neuhof, *The Transplantation of Tissues*, New York (1923)

⁴ Shawan *Amer Jour Med Sci* 167 503 (1919), Landsteiner *Science* 73 403 (1931), Loeb *loc cit*, Todd *Proc Roy Soc B* 20 (1930)

⁵ *Loc cit* However see Kozelka *Physiol Zool* 6 159 (1933), Haddow *Jour Path and Bact* 39 345 (1934)

all other individuals in the same species, which were not blood relatives

In man, most observations on tissue transplantation have been concerned with skin grafting from one individual to another. That the blood groups play a part in the failure of these grafts is suggested by the observation that the group specific substances are present in practically every tissue of the body. Some surgeons (Shawan,⁶ Kubanyi,⁷ Baldwin,⁸ Schwarzmann,⁹ etc.) claim that there actually is a definite connection between the results of grafts and compatibility of the blood. Shawan even states that he had 100 per cent successful grafts when the donors of the grafts were selected on the basis of compatibility of the blood. On the other hand, many surgeons (Lexer,¹⁰ Schone,¹¹ Holman,¹² etc.) absolutely deny that skin grafting can be successfully performed between two different human beings, even when the donors are selected by blood grouping tests. These conflicting opinions can perhaps be explained by differences in technic or by the fact that grafts which take at first may finally be cast off, even after a long period of time¹³ so that unless observations are carried out over an extended period it may incorrectly be assumed that the transplant will permanently survive.

One may conclude from these observations that whereas the blood group differences may be of some importance, they are certainly not the sole deciding factor,¹⁴ otherwise, the problem of tissue transplantation in man would be entirely solved. Furthermore, in those animals in which isoagglutination is absent, homotransplantation should be more successful than it actually is.

However, tissue grafting cannot be considered strictly analogous to blood transfusion. In blood transfusion, the sole requirement is that the transfused blood survive and function for a limited period of time whereas in grafting the tissue must survive indefinitely.

For this reason, it is to be expected that in addition to the differences demonstrable with isoagglutinins, others, even when of a minor order, will influence the outcome of grafting.¹⁵

⁶ *Loc cit*

⁷ *Arch f klin Chir* 129: 644 (1924)

⁸ *Med Record* 98: 686 (1920)

⁹ *Zentralbl. Gynak* 52: 1593 (1928)

¹⁰ Lexer, *Die freien Transplantation* Stuttgart (1919)

¹¹ *Loc cit*

¹² *Surg, Gyn and Obst* 38: 100 (1924)

¹³ Schone, *loc cit*, Lexer, *Arch f klin Chir* 95: 827 (1911)

¹⁴ Jelanski believes that even if sloughing of the grafted tissue is unavoidable blood grouping may be of some value since with compatible transplants sloughing seems to be delayed and a more favorable result may be obtained because cell proliferation from the edges of the wound is enhanced [*Nov. Chirurgisches Archiv* 3: 59b (1923)]

¹⁵ Transplantation of tissue from one member of a pair of identical twins to the

Direct evidence of the importance of individual blood differences for transplantation of tumors has recently been furnished by Gorer.¹⁶ This investigator studied three pure lines of mice, obtained by brother-sister matings for twenty generations (cf page 358). A sarcoma which arose in one of the pure lines was found to be transferable to all other members of the line and certain hybrids derived from it, but not to unrelated mice. Genetic investigation showed that two (possibly three) dominant genes had to be present in the host or the tumor would regress. One of the genes corresponded with that determining the presence of an agglutinin present in the blood of normal mice of the strain in which the tumor arose. Further evidence of the relation of this agglutinin to the survival of the transplants was the appearance of isoagglutinins for blood containing the agglutinin, in the sera of mice in which the tumor had recently regressed. However, while in Gorer's experiments individual blood differences affected the survival of the transplant, there were other genetic factors of an unknown nature which likewise played an important role.

Blood Groups and Pathology It is well established that isoagglutination (and also isohemolysis) is in no way dependent upon disease. But many writers have asserted that persons belonging to certain blood groups are more likely to contract certain diseases, and that the course of the disease is different in the various groups. If this opinion were correct, one would expect to find that the distribution of the blood groups among individuals suffering from the disease in question differs significantly from the distribution in the general population. Reliable investigators¹⁷ have failed to find any evidence of such a correlation (cf table 87), and believe it unlikely that the blood groups of an individual does influence the occurrence or course of any disease in that individual.

In the statistics which have been alleged to demonstrate differences in the blood group distribution among healthy individuals and among individuals suffering from certain diseases in many cases the number of individuals examined was small so that the differences found were

other is equivalent to autotransplantation and should therefore *a priori* be successful. Such a case has been reported by Bauer [*Bruns' Beitr zur klin Chir* 141:442 (1927)].

¹⁶ *Jour Path and Bact* 44:691 (1937) *ibid* 47:231 (1938). Also see the recent report by Lumsden [*Amer Jour Cancer* 32:393 (1938)] describing transplantation experiments with Jensen rat sarcoma in rats.

¹⁷ Snyder *Blood Grouping in Relation to Legal and Clinical Medicine*, Baltimore (1929). Sievers *Rinskä Lakarettallsk Handl* 71:636 (1929). Streng and Rytö *Acta Soc med Fenn "Duodecim"* 8:6 (1927). Amzel and Halber *Ztschr f Immunitäts* 42:89 (1925). Salek *Ztschr f Immunitäts* 74:280 (1932). Moureau *Rev belge des Sci Méd* Vol VII (1935). Mustakallio *Acta Soc Med Fenn Duodecim* 20A:No 2 (1937) etc.

TABLE 87

DISTRIBUTION OF THE BLOOD GROUPS IN HEALTH AND IN SOME PATHOLOGIC CONDITIONS (After Snyder)

Condition	Number Studied	Per Cent in Group			
		O	A	B	AB
Normal	20 000	45	41	10	4
Dementia praecox	200	45	42	9	4
Epilepsy	150	48	40	10	2
Feeble mindedness	300	44	42	10	4
Malaria	100	47	30	18	5
Syphilis	100	44	42	8	6
Migraine	500	47	30	7	6

not significant (cf page 170). In other cases evidently the technic was at fault, as in the studies of Feldmann and Elmanowitsch¹¹ who found a high percentage of group AB in 815 cases of mental disease (in parietic patients as high as 33 per cent). In some studies where apparently a reliable technic was used the differences found though small were statistically significant. Thus according to the results obtained by certain Russian investigators (cf table 88), it would seem that individuals of group O are less likely to contract malaria than individuals of group AB (or group B). However, the statistical correlation observed may also be explained in the following manner:

It has been shown that the distribution of the blood groups and the susceptibility to certain diseases varies in different races. Let us sup-

TABLE 88
BLOOD GROUPS AND MALARIA (RUSSIAN AUTHORS)
(After Lattes)

Condition	Number Studied	Per Cent in Group			
		O	A	B	AB
Normal	13 484	33.5	37.9	21.3	7.3
Malaria	4 397	27.5	37.9	22.8	11.8

pose that a certain race having a higher incidence of the disease in question and having a high frequency of group AB (and group B) is mixed with a race relatively resistant to the disease and with a relatively low frequency of group AB (and group B). In the mixed popula-

¹¹ *Med Biol Jour* 12: 125 (1925)

on, it will be found that the frequency of group AB (and group B) is higher among diseased individuals than in the general population. Thus a correlation would obtain, just as in the investigations cited above. If the resistant race contained mainly blue-eyed individuals and the susceptible race mainly brown eyed individuals, it would be found

TABLE 89
AGGLUTINOGENS M AND N IN MENTAL DISEASE
(After Herman and Derby)

Diagnosis		Frequencies of Blood Types*			Number of Individuals Examined
		M	N	MN	
Control Series		570 29 9±0 7	385 20 2±0 6	950 49 9±0 8	1905
Dementia Praecox	Observed	28 2±1 4	22 2±1 2	49 6±1 5	508
	Difference	-1 7±1 6	+2 0±1 4	-0 3±1 7	
Manic Depressive	Observed	32 9±2 3	21 8±2 0	45 2±2 4	188
	Difference	+3 0±2 4	+1 6±2 1	-4 7±2 6	
General Paresis	Observed	31 1±1 9	21 2±1 7	47 6±2 0	273
	Difference	+1 1±2 0	+1 0±1 8	-2 2±2 2	
Cerebral Arterio- sclerosis with Psychosis	Observed	27 7±2 0	22 3±1 9	50 0±2 3	224
	Difference	-2 2±2 1	+2 1±1 0	+0 1±2 5	
Involutional Psychosis	Observed	30 6±2 5	16 9±2 0	52 5±2 7	160
	Difference	+0 7±2 6	-3 3±2 1	+2 6±2 9	
Unclassified	Observed	28 2±1 4	23 4±1 2	48 4±1 5	496
	Difference	-1 7±1 6	+3 2±1 3	-1 5±1 7	
Mental Dis- ease in general	Observed	29 3±0 7	21 9±0 7	48 8±0 8	1849
	Difference	-0 6±1 0	+1 7±0 9	-1 1±1 1	

* Figure following ± sign represents probable error.

in the mixed population that more brown eyed people contract malaria than blue-eyed people. No one would conclude, however, that for this reason the color of the eyes has a direct relationship to the susceptibility of an individual to malaria.

It is significant that whenever a supposed correlation between blood groups and diseases has been reported, the observations could not be

confirmed by later investigators. Thus, for general paresis one author reported an increase in group AB,¹⁹ a second an increase in group B and group AB,²⁰ and a third a predominance of group A,²¹ whereas the vast majority of authors²² failed to find any difference at all.

In a recent study by Herman and Derby²³ in which the distribution of the blood groups in 1849 patients with mental disease was compared with the distribution of the groups in control series of 1444 individuals, no significant difference in the distribution of the blood groups was found either in general paresis or in any other mental disease or in the combined series of patients with mental disease in general. In this connection it might be mentioned that the alleged correlation recently reported between blood groups and susceptibility to poliomyelitis has been criticized by Schiff.²⁴ Studies of this sort are still being made and a perusal of the literature of the past few years reveals attempts to establish a relationship between blood groups and duration of life or diseases as diverse as dental caries, psychosis, skin diseases, bone and joint tuberculosis, etc.

In recent years these investigations have been extended to the distribution of the agglutinogens M and N in health and disease. Herman and Derby²⁵ found no correlation between mental disease and the M N types, whether the various mental diseases were considered individually or together (cf. table 89). In a study by Moureau,²⁶ in which 3100 normal individuals and almost 1000 sick persons were tested, there was no correlation between the M N types and tuberculosis, syphilis, cancer or mental disease, and Mustakallio,²⁷ who tested the bloods of 2424 normal and 4389 sick persons, observed no significant difference in the distribution of the M N types in a wide variety of diseases. Moureau and Mustakallio, moreover, found no significant difference in the distribution of the types in the two sexes (confirming Landsteiner and Levine's original observations) or in different age groups. In the series examined by Mustakallio, tests were also made for the subgroups of group A and group AB, and here again the findings were negative.

¹⁹ Wilczkowski, *Klin Woch* 6: 168 (1927).

²⁰ Gundel, *Munch med Woch* 75: 1337 (1928). Perkel and Israelson, *Dermatol Ztschr* 54: 261 (1928).

²¹ Kaševarov, *Psych Clin Univ* No 2: 144 (1928).

²² For literature see Thomas and Hewitt, *Jour Mental Sci* 85: 66 (1939).

²³ *Jour Immunol* 33: 87 (1937). With regard to the titer of the isoagglutinins in mental diseases (cf. page 375) see Suominen, *Acta Soc Med Fenn* 14: No 2 (1932). Thomas and Hewitt, *loc cit*.

²⁴ *Klin Woch* 14: 786 (1935).

²⁵ *Jour Immunol* 33: 87 (1937). Also see Thomas and Hewitt, *Jour Mental Science* 85: 667 (1939).

²⁶ *Rev belge des Sci Med* 7 (1935).

²⁷ *Acta Soc Med Fenn Duodecim* 20: No 2 (1937). Also see Cardozo, *Arch Int Med* 60: 623 (1937).

Heterospecific Pregnancy A number of the earlier workers on blood grouping suggested that 'heterospecific pregnancy,' that is, blood group incompatibility between mother and fetus might give rise to morbid conditions in the mother and infant, but these theories were abandoned when statistical analysis of the available data failed to support them. Since some of these ideas have recently been revived by Levine²⁸ it was considered desirable to review them here.

As long ago as 1905 Dienst²⁹ pointed out the similarity between the symptoms and post mortem findings in eclampsia and in hemolytic transfusion reactions. He suggested that the abrupt onset of symptoms in eclampsia was due to the sudden formation of a gross defect in the placenta and the resulting transfusion into the mother of incompatible fetal blood. However Dienst³⁰ himself abandoned this hypothesis when he found that eclampsia also occurred where the bloods of mother and fetus were compatible. The theory was then forgotten until taken up again by McQuarrie³¹ in 1923 who proposed it as an explanation not only of eclampsia but also of toxemia in pregnancy. Ottenberg³² developed the hypothesis further by proposing that in cases of toxemia a more or less continuous leakage of fetal blood occurs during pregnancy into the maternal circulation with resulting isoimmunization. However the theory that heterospecific pregnancy was responsible for eclampsia or toxemia in the mother was abandoned for lack of evidence and the etiology of these conditions remains unknown to the present time.³³

In view of the permeability of the human placenta to maternal antibodies (cf page 25), Hirszfeld³⁴ suggested that in heterospecific pregnancy the fetus *in utero* might be damaged in this way. On the other hand, certain mechanisms tend to protect the fetus from the maternal isoantibodies, such as the low sensitivity of the fetal erythrocytes³⁵ to the action of the isoantibodies (cf page 21), and the presence of group substances in the amniotic fluid and organs (at least of secretors). As already mentioned, moreover, the placenta seems to be a neutral zone (cf page 273) as far as group substances are concerned and Hirszfeld and Zborowski³⁶ have noted a marked drop in the titer

²⁸ *West Jour Surg Obst and Gyn* 50 468 (1942)

²⁹ *Zentrabl f Gynak* No 21 651 (1905)

³⁰ *Arch f Gynak* 86 314 (1908)

³¹ *Bull Johns Hopkins Hosp* 34 51 (1923)

³² *Jour Amer Med Assoc* 81 295 (1923)

³³ In the hypothesis discussed only the reaction of the maternal isoantibodies with the fetal blood was considered. The possibility that fetal isoantibodies might act on the maternal blood cells is excluded since the capacity to form antibodies is not developed during fetal life all antibodies present at birth being derived from the mother by filtration through the placenta (cf page 23)

³⁴ *Konstitutionserologie und Blutgruppenforschung* p 78 (1928)

³⁵ With potent antisera however the presence of agglutinogens in fetal blood is readily demonstrated as early as the fourth or fifth month of intrauterine life

³⁶ *Klin Woch* 4 1152 (1925) *ibid* 5 77 (1926)

of the maternal isoagglutinins (or even complete absence of isoagglutinins) in retroplacental blood

As evidence that heterospecific pregnancies may produce morbid processes in the child, Hirszfeld and Zborowski presented data purporting to show that in such pregnancies the average weight of the new born child is less than in homospecific pregnancy. Furthermore, Hirszfeld claimed that contrary to what would be expected on the basis of simple hereditary probabilities, the mother's group occurred more frequently in the child than the father's. Similarly disharmony of the blood groups of man and wife has been proposed as a cause of sterility or diminished fertility. In a critical statistical analysis of the available data, Koller³ demonstrated that the results are not adequate to prove the hypothesis that heterospecific pregnancy has harmful effects on the fetus. Recently, Hirszfeld¹⁸ himself has retracted his hypothesis, stating that while his earlier statistics appeared to support his ideas, those accumulated since 1934 have failed to do so.

It has been calculated that if the effect of heterospecific pregnancy were sufficiently pronounced to bring about a noticeable effect in a single generation, then in the course of thousands of generations all individuals should eventually belong to the same blood group.³⁹ The fact that with only a few exceptions all four groups are represented in populations in different parts of the world (cf. table 6) contradicts this assumption. To be sure, it is possible that a very small number of fetal deaths is due to this mechanism, but if so these must be too few to be detectable⁴⁰ by the analysis of ordinary family data, though sufficient to have had an appreciable effect on the distribution of the blood groups in man over a long period of time (cf. page 372). On the other hand, the discrepancies noted in the ordinary family data can be ascribed more reasonably to chance (considering, for example, that as many as one out of 64 families with 6 children in which the father belongs to group A and the mother to group O would be expected to have only group O children) errors in technique or illegitimacy.⁴¹

Apart from the question whether heterospecific pregnancy can produce pathological conditions in the mother and child, it has been demonstrated by Jonsson⁴² that isoimmunization actually can take place in

³ *Ztschr. f. Rassenphysiol.* 3: 121 (1931).

¹⁸ Hirszfeld, *Les Groupes Sanguines*, p. 18 (1938).

³⁹ Cf. Koller, *loc. cit.*

⁴⁰ One abortion is said to occur for every 2 or 3 live births [Rock, *Abortion in Medical Progress Annual*, p. 15, Charles C. Thomas (1940)]. Even if one assumes that as many as one in 10,000 or 1 in 1,000 pregnancies are affected by the groups of the mother and child, this would hardly be noticeable in a statistical analysis of abortion cases. In this connection, even though it is known (cf. page 369) that isoimmunization against the Rh factor can affect the viability of the fetus, this does not disturb the distribution of the Rh agglutinin in the offspring sufficiently to be demonstrable in ordinary heredity studies (cf. table 50).

⁴¹ Cf. Koller, *loc. cit.*

⁴² *Acta Path. et Microbiol. Scand.* 13: 424 (1936).

such cases. For example, he found that the titer of the α agglutinin was higher in group O mothers who had recently given birth to group A infants than in those mothers bearing group O or group B infants.

Rh Factor in Erythroblastosis Fetalis While the idea that isoimmunization in pregnancy plays a rôle in the pathogenesis of erythroblastosis fetal⁴³ is not entirely new, it was not until very recently that evidence by Levine and his collaborators⁴⁴ was obtained which established the accuracy of the theory.

Ottenberg⁴⁵ attempted to establish a relationship between heterospecific pregnancy and icterus gravis neonatorum, he suggested that maternal isoantibodies (natural or increased in titer by isoimmunization with incompatible fetal blood) filtered back through the placenta and destroyed the fetus's erythrocytes. This hypothesis was not further pursued when no correlation could be established between blood group incompatibility and the disease. More recently Darrow reported serological differences between the hemoglobins from fetal and adult erythrocytes and then suggested that isoimmunization of the mother to the fetal hemoglobin might be the deciding factor.⁴⁶ However Darrow was unable to present any direct evidence to support her hypothesis.

Shortly after the Rh factor was discovered by Landsteiner and Wiener, and after the capacity of this factor to stimulate isoantibody formation in man was established by Wiener and Peters (cf. page 268) it was found by Levine and his coworkers that a correlation existed between the occurrence of intragroup transfusion reactions in post-partum cases due to the Rh factor and complications in pregnancy,⁴⁷ in particular erythroblastosis in the fetus or newborn.⁴⁸ Then, in an extensive statistical investigation, Levine and his collaborators⁴⁹ succeeded in compiling convincing evidence that the Rh factor played an essential rôle in the pathogenesis of the great majority of the cases of erythroblastosis.

According to the theory of Levine and his associates, in the typical case, the mother is Rh negative, the father Rh positive and the fetus Rh positive, the latter having inherited the Rh factor from the father. Due presumably to some defect in the placenta, fetal blood enters the

⁴³ This syndrome includes a group of related conditions namely fetal hydrops, icterus gravis neonatorum and hemolytic anemia of the newborn. In addition there are a certain number of stillborn infants which show the pathologic changes characteristic of the disease.

⁴⁴ Levine, Katzin and Burnham *Jour Amer Med Assoc* 116: 825 (1941).

⁴⁵ *Loc cit*.

⁴⁶ *Arch Path* 25: 378 (1938).

⁴⁷ Levine and Katzin *Proc Soc Exp Biol and Med* 45: 343 (1940).

⁴⁸ Levine, Katzin and Burnham *Jour Amer Med Assoc* 116: 825 (1941). Burnham *Amer Jour Obst and Gyn* 42: 389 (1941).

⁴⁹ Levine et al. *Science* 94: 3,1 (1941), *Amer Jour Obst and Gyn* 42: 925 (1941).

circulation of the mother and incites the production of Rh isoantibodies. These in turn filter through the placenta into the fetal circulation. In severe cases the resulting destruction of fetal blood cells is so extensive that death of the fetus *in utero* occurs, and this manifests itself as fetal hydrops or a stillbirth. In many cases, however, it seems that the Rh antibodies are in some manner prevented from acting on the fetal blood cells and destruction of the infant's blood becomes manifest only after birth. The possibility has been mentioned⁴ that anti Rh isoantibodies can also be transmitted to the nursing infant through the colostrum and milk.⁵ The intensity of the symptoms is variable. In fulmi-

TABLE 90

THE Rh FACTOR AND ERYTHROBLASTOSIS FETALIS

(From Levine Yearbook of Pathology and Immunology for 1941 page 508)

	Per cent Rh positive	Per cent Rh negative
Random population (1 035 individuals)	86	14
153 mothers of infants with erythroblastosis	8	92
89 husbands of Rh negative mothers	100	0
76 infants with erythroblastosis	100	0

nating cases death may occur within a few hours. In milder cases the hemolysis terminates before much damage is done and complete spontaneous recovery occurs. In some cases, the life of the infant has been saved by tiding it over with blood transfusions of Rh negative blood until the anti Rh isoantibodies were eliminated from its body⁶ (cf. page 87).

The most convincing evidence of the accuracy of the theory is the statistical data concerning the distribution of the Rh factor in mothers of erythroblastotic infants (cf. table 90). Among these women Levine and his collaborators found as many as 92 per cent who gave negative reactions when tested with human anti Rh sera in contrast to only 14 per cent negative reactions among individuals from a random series. Moreover, the husbands and infants of the Rh negative mothers were uniformly Rh positive. Levine concludes that at least 90 per cent of the cases of erythroblastosis can be traced to isoimmunization to the Rh factor, the remainder he suggests may be due to isoimmunization to other blood factors.

As has already been pointed out (cf. page 252) human anti Rh sera do not all

⁴ Cf. Witebsky et al. *Proc. Soc. Exp. Biol. and Med.* 49: 179 (1942). Wiener *Yearbook of Pathology and Immunology for 1941* p. 503.

⁵ Levine et al. *loc. cit.*, Wiener, unpublished observations.

give identical reactions the three most frequent types giving 13 15 and 30 per cent negative reactions, respectively Levine classed the mother as Rh negative in his table if she gave a negative reaction with any one of these three sera provided the husband was Rh positive. However the incidence of Rh negative bloods in the control series was taken to be 14 per cent while with corresponding anti Rh sera 85 to 90 per cent of the mothers of erythroblastotic infants gave negative reactions. On this basis 85 to 90 per cent (rather than 92 per cent) of the cases of erythroblastosis should be attributed to the Rh factor unless one assumes as Levine does that isoimmunization may occur when mother and infant belong to different subdivisions of the Rh positive type. Wiener⁴² has tested 43 mothers of erythroblastotic infants and found 37 or 87 per cent to be Rh negative as contrasted with about 15 per cent Rh negative individuals in the general population.

TABLE 91

INCIDENCE OF ANTI Rh AGGLUTININS IN 141 Rh NEGATIVE MOTHERS OF
ERYTHROBLASTOTIC INFANTS
[From Levine et al, *Amer Jour Obst and Gyn* 42 925 (1941)]

Interval after Last Delivery of an Affected Infant	Agglutinins Present	Agglutinins Absent
2 months postpartum	33	37
2 months to 1 year postpartum	5	15
1 year or longer postpartum	2	39
During next pregnancy	2	5
No data	0	3
Totals	42	99

Further proof of the rôle of isoimmunization in erythroblastosis was obtained by Levine and his associates by testing the sera of Rh negative mothers for anti Rh isoagglutinins. As is to be expected (cf table 91), the chances of finding such immune isoantibodies in the mother's serum are better if the tests are carried out soon after the delivery of the erythroblastotic infant, though in at least 2 cases the agglutinins were detectable for periods up to 2 years. While anti Rh antibodies can frequently be demonstrated in the mothers' sera, it is important to note that in more than half the cases no such antibodies are detectable, and in the great majority of the cases with anti Rh isoantibodies these are of low titer.⁴³ This may seem surprising in view of the severity of the symptoms in the infant, but as has already been pointed out (cf page 66), a similar situation exists in most of the intragroup hemolytic transfusion reactions due to the Rh factor. A possible explanation is that the anti Rh isoantibodies have a tendency to attach themselves

⁴² *Amer Jour Clin Path* 12 302 (1942) and unpublished data.

⁴³ Levine et al *loc cit*. Wiener unpublished observations, Boorman Dodd and Mollison *Brit Med Jour* 2 569 (1942).

to tissue cells, so that the amount of circulating antibodies may not furnish a reliable index of the total amount of antibodies in the body.

Levine's theory explains why in some families all pregnancies but the first terminate with infants having erythroblastosis, while in other families the disease occurs more irregularly. If the father is homozygous (genotype $RhRh$) all the children would be Rh positive and therefore capable of developing the disease (cf page 249). The reason why in some of these families the first one or two infants escape the disease may be that in these mothers one or more pregnancies are necessary before sufficient isoimmunization against the Rh factor occurs. When the father is heterozygous (genotype $Rhrh$), it is clear that only half the children will be susceptible to the mother's anti Rh isoantibodies and in such families the incidence of the disease will be lower.

Since as many as 13 per cent of all families (mother Rh— and father Rh+) offer an opportunity for isoimmunization in pregnancy against the Rh factor, one might expect a similar high incidence of erythroblastosis. According to Javert,³⁴ however, only about 1 in every 400 newborn infants suffers from this disease. The probable reason for this as well as the low incidence of intragroup hemolytic reactions due to the Rh factor in patients receiving repeated transfusions (cf page 117), is that not every Rh negative individual will produce antibodies to the Rh factor.³⁵

Since the agglutinogens A and B are far better antigens in man than the Rh factor (cf page 267), it may seem surprising at first that heterospecific pregnancy appears to play no rôle in erythroblastosis. As has already been pointed out, however, the presence of group substances A and B in the amniotic fluid and in the organs and secretions of the fetus may serve to protect it from the maternal anti A and anti B isoantibodies. The Rh factor, on the other hand, appears to be limited to the erythrocytes (cf page 294), so that such a protective mechanism cannot operate. The 10 to 15 per cent erythroblastotic infants with Rh positive mothers might be traced to other blood factors which like Rh, are capable of stimulating isoantibody formation occasionally in man.³⁶

It may be of interest to speculate what influence isoimmunization in pregnancy may have had on the present and will have on the future distribution of the Rh

³⁴ *Amer Jour Obst and Gyn* 34 1042 (1937)

³⁵ Cf Wiener *Amer Jour Clin Path* 12 241 (1942)

³⁶ The factors in question would be expected to be less antigenic than Rh. Levine has presented evidence to show that the factor Hr present in Rh negative blood (cf page 254) and the factor found by Levine and Polayes (cf page 270) may also account for some of the exceptional cases. [Cf Wiener *Amer Jour Clin Path* 12 302 (1942)]

factor in man. At first it might seem that the elimination of occasional Rh positive fetuses and infants should tend to favor Rh negative individuals in the long run. However, the Rh positive fetuses affected are all heterozygous so that an equal number of *Rh* and *rh* genes would be lost in each generation. Accordingly in populations where the frequencies of the two genes are equal this process would not disturb the distribution of the genes. In populations where the frequencies are unequal the less frequent gene would be affected to a relatively greater extent, so that the more frequent gene whether *Rh* or *rh* would tend to predominate more and more as time passed.

With regard to the problem how the present distributions of the Rh factor arose two main possibilities may be considered analogous to those suggested to explain the distributions of the four blood groups: (1) that the present frequencies resulted mainly from repeated mutations from the Rh positive type to the Rh negative type (or vice versa); (2) that two or more races, some predominantly Rh negative others predominantly Rh positive, were established by isolation and inbreeding and that the present distributions resulted from crosses between these populations. Wiener^{35a} believes the former theory to be unlikely because while this might account for the occasional occurrence of Rh-negative individuals among American Indians and Chinese, to explain the distribution in white individuals one would have to postulate an improbably high rate of mutation, especially since isoimmunization in pregnancy would tend to counteract the effect of such mutations for the reason pointed out above.

Studies on Linkage between Blood Groups and Disease. Since morphological characters and susceptibility to certain diseases are hereditary in nature, and since the blood groups are also inherited, a number of these qualities will necessarily be linked to blood groups in their heredity. It is obvious, however, that since man has twenty-four pairs of chromosomes, *a priori*, the chance that the genes for a given pathological condition (or any given trait) are located in the same pair of chromosomes as the genes *A*, *B*, and *O* is small. Consequently, a large number of investigations is needed before a case of linkage will be found.

A number of authors have tried to demonstrate linkage by studying the distribution of the blood groups among individuals possessing the character under consideration. This method will not demonstrate linkage, however, for even if linkage exists (excluding the very improbable case of complete linkage), as a result of random intermarriage, the distribution of the blood groups will be the same among individuals possessing the trait as in the general population. To study linkage, it is necessary to accumulate statistics in families, and as yet the number of such studies is limited. Snyder³⁷ made a careful analysis of all the data available up to 1931 and concluded that no case of linkage with the blood groups had been discovered, although investigations had been made on its possible linkage to eye color, direction of occipital hair

^{35a} Wiener, *Science* 96: 407 (1942).

³⁷ *Eugenical News* 16: 177 (1931); *Ztschr. f. Immunitäts* 49: 464 (1926).

wborl, polydactylism,⁵⁸ migraine,⁵⁹ atopic hypersensitiveness,⁶⁰ and susceptibility to diphtheria.⁶¹ This is not surprising for only one case of linkage could be expected for every 24 traits studied.

The chances of discovering somatic linkage with serological blood properties are much greater if tests made not only for A B, but also for M N and Rh, since the latter serve as markers for two additional pairs of chromosomes (cf page 252).

At one time it was believed that it was necessary to examine three generations in order to study linkage properly in man, but Bernstein⁶² and Wiener⁶³ independently devised methods whereby conclusions can be drawn from studies of only two generations (cf pages 242 and 252). This has simplified the task of collecting the necessary data and has led to further investigations in the field. Thus, using modern statistical methods, the author has shown that there is no linkage in heredity between the agglutinin P, the blood groups, the M N types or eye color. Hogben⁶⁴ could not establish linkage between the four blood groups, taste blindness, Friedrich's ataxia, or major brachydactyly in man, and Zieve, Wiener and Fries⁶⁵ found no evidence of linkage between allergic disease, blood groups, M N types or eye color.

Studies on sex linkage are more readily made than studies on "somatic" linkage, i.e. in chromosomes other than the sex chromosomes. Thus, more than fifteen sex linked conditions are known at the present time,⁶⁶ but not a single case of somatic linkage. For this reason the discovery of cases of linkage with blood groups will be of importance for human genetics. To clinical medicine, such studies hold little promise of any immediate findings of importance, for only in cases of complete or very close linkage, which are extremely rare, would it be possible to make definite predictions of clinical value. Nevertheless work along this line should be continued because it will increase our knowledge of human genetics, and ultimately may become of some practical value.

Haldane⁶⁷ has compared the methods of detecting and measuring linkage de-

⁵⁸ Thomsen *Handbuch der Blutgruppenkunde*, p. 260 (1932).

⁵⁹ Snyder *loc cit*.

⁶⁰ Levine *Jour Immunol* 11: 283 (1926).

⁶¹ Hirsfeld and Brokman *Jour Immunol* 9: 5-1 (1924).

⁶² Zisch *J. indukt Abstamm u Vererbungs* 57: 113 (1931). Bernstein and Machol *Proc Roy Soc B* 117: 63 (1935).

⁶³ *Genetics* 17: 335 (1932).

⁶⁴ *Jour Genetics* 31: 353 (1935).

⁶⁵ *Ann Eugenics* 7: 163 (1936).

⁶⁶ It is of course futile to attempt to establish linkage between blood groups and a sex linked trait since the blood groups are not sex linked. Yet Kubányi [*Álm H och* p. 321 (1926) p. 597 (1931)] and others have attempted to demonstrate linkage between blood groups and hemophilia (cf page 166).

⁶⁷ *Amer Nat* 68: 286 (1934).

vised by Bernstein and Wiener, and found the formulae of the former to be slightly more efficient. On the other hand Wiener's method has the advantage that it is somewhat simpler to apply and the slightly lower efficiency of the formulae is more than counter balanced by the improvement obtained by excluding children of indeterminate genotype (cf page 242). Carrying these principles further Haldane⁶⁸ has derived formulae by means of which it is possible to extract maximal information with regard to linkage from matings of various types. Further improvements have been devised by Fisher⁶⁹ and by Finney.⁷⁰ Appropriate tables to facilitate the estimation of linkage in heredity studies can be found in the papers by Bernstein, Wiener and Finney.

More recently Penrose⁷¹ and Burks⁷² have independently suggested a method whereby it is theoretically possible to detect linkage in man from studies on only a single generation. The principle of this method is to divide the siblings in each family by pairs into four classes with regard to the two characters being tested namely like-like, like-unlike, unlike-like and unlike-unlike. In this way a family with 8 children would yield 28 sibling pairs to be analyzed. The results are entered in a four fold table and the deviation from the distribution expected on the assumption of random assortment is determined by the chi square method. If a statistically significant deviation is obtained then this is attributed to linkage between the genes for the two characters.

In the opinion of the author while the method of Penrose and Burks is correct in principle it is of little practical value for the detection of linkage for the following reasons. Since the phenotypes of the parents are not ascertained children from all families are necessarily included in the analysis including those families which cannot possibly yield any information as to linkage. Accordingly data containing significant information is swamped by indifferent material. For example in the heredity study of Landsteiner and Wiener⁷³ there were only two families which could be used for determining the linkage relations of Rh and A B but the information was extracted without difficulty by the direct analysis of these families. If the same study had been carried out by the sibling method 437 sibling pairs from all the families would have had to be analyzed. It seems obvious that it takes less effort to test parents as well as children in each family than to make the calculations required by the sibling method and in addition the results obtained by the latter method are bound to be far less reliable. Thus it is more likely that any deviation obtained in the sibling method will be due to the operation of chance than to the presence of linkage considering that the pertinent data will make up only a small part of the total material. Nevertheless attempts have been made recently to apply the method for the detection of linkage between blood group genes and genes for other traits such as mid digital hair, oval erythrocytes, etc.⁷⁴

*Isaagglutinins in Health and Disease*⁷⁵ Whereas the blood group of

⁶⁸ *Ann. Eng.* 6: 26 (1936).

⁶⁹ *Ann. Eng.* 6: 186 (1935); *ibid.* 6: 339 (1935).

⁷⁰ *Ann. Eng.* 10: 171 (1940); *ibid.* 11: 10 (1941); *ibid.* 11: 115 (1941).

⁷¹ *Ann. Eng.* 6: 133 (1935); *ibid.* 8: 233 (1938).

⁷² *Yearbook Carnegie Inst. Wash.* 35: 312 (1937); *Proc. Nat. Acad. Sci.* 24: 312 (1938).

⁷³ *Jour. Exp. Med.* 74: 309 (1941).

⁷⁴ Burks, *Genetics* 26: 223 (1941); Boyd and Boyd, *Ann. Eng.* 11: 1 (1941); Snyder, Baxter and Kneely, *Jour. Hered.* 32: 23 (1941).

⁷⁵ For a review of this subject see Suominen, *Acta Soc. Med. Fenn.* 'Duo decim' 14: No. 2 (1932).

any individual can be determined at birth and remains constant throughout life, the isoagglutinin content of the serum is less constant. Aside from variations in the potency of the isoagglutinins among different individuals, apparently at least partly constitutional in nature, the titer of the isoagglutinins depends in large measure on the age of the individual (cf page 22). Moreover, the titer may be increased somewhat in acute infections or by other non specific stimuli whereas in certain diseases such as chronic leukemia and severe anemias the titer may be lowered. In addition the isoantibody titer may occasionally undergo spontaneous changes without ascertainable cause. On the other hand, there is no significant difference between the average isoagglutinin titers in the two sexes, and the isoagglutinin titer usually tends to remain constant in one and the same individual for long periods of time (years), despite intervening organic disease, surgery, etc.

Marked rises in the titer of the isoagglutinins occur following transfusions of blood of an incompatible group, while the titer remains unchanged after homologous group transfusions (cf page 123). The isoagglutinin titer has also been reported to attain very high values in heterospecific pregnancy (cf page 368). Similar observations have been reported following plasma transfusions and these have been attributed to the specific immunizing effect of the group-substances in solution in the plasma.⁶ However, since the quantities of group substances in plasma are small (cf page 285), it is possible that the presence of red cell stromata may have contributed to the immunization effect. Davidsohn⁷ has reported the occurrence of very high titers of isoagglutinins in patients with horse serum disease. That the rise in titer was stimulated by the injection of horse serum was established by comparing the titer before and after the injections. A rise in titer from 10 to 200 was not unusual, and in one patient the titer rose thousand fold. Since it is difficult at times to secure isoagglutinating sera of high titers for the purpose of blood grouping, Davidsohn remarks that it may prove practical to prepare typing sera from the blood of patients with horse serum disease.

Blood Groups and Twin Studies Since monovular or identical twins have the same genotype, in contrast to bivular twins, who are genetically no more closely related than ordinary brothers and sisters, studies on twins have been utilized for comparing the effects of nature and nurture, and for solving problems in human heredity. An important prerequisite for such investigations is the use of suitable criteria for distinguishing the two sorts of twins. In general, if a pair of twins

⁶ Aubert, Boorman and Dodd *Jour Path and Bact* 54: 89 (1942).

⁷ *Amer Jour Clin Path* 8: 179 (1938). Cf Wiener and Kosofsky *Jour Immunol* 41: 413 (1941).

differs in sex or any other trait, the hereditary nature of which is established, they cannot possibly be monovular. On the other hand, the fact that two twins resemble each other with regard to a large number of hereditary characters does not absolutely prove them to be identical, though this would become highly probable. Aside from sex, the only characters shared by all human beings and transmitted in a simple fashion according to the Mendelian laws are the agglutinogens in the erythrocytes. Accordingly, blood tests should be included routinely as one of the criteria of zygosity in all investigations on twins.

The chance that a pair of fraternal twins will differ with respect to a particular hereditary trait will vary with its distribution in the general population. Wiener and Leff⁷⁸ have derived general formulae for the chances of establishing the non identity of bi-ovular twins that can be applied to all the common types of Mendelian transmission. These investigators have calculated that for a population such as that present in New York City almost two thirds of all like sexed fraternal twins can be identified as such by tests for A_1 , A_2 , B and MN.⁷⁹ The chances can be further increased of course by making tests for the secreting types and the Rh factor. For example the writer was recently consulted by Dr. Donald Bauer concerning a pair of presumably identical twins, one with situs inversus, the other with situs solitus. While one twin was found to belong to group A_1B type MN Rh negative and was a non secretor, the other proved to belong to group A_1B type N Rh negative and was a secretor, contradicting the assumption that the twins were monovular.

Blood Groups and Therapeutic Malaria. Infection with malaria is frequently used in the treatment of general paresis. The usual procedure followed is to take 5 c.c. of blood from a patient already infected with the malarial parasite, and to inject this blood immediately into the vein of the patient to be treated. Wendleberger⁸⁰ was the first to observe that when incompatible blood is injected the average incubation period is twice as long as when compatible blood is given. The accuracy of this observation has been established beyond doubt by a number of independent investigators.⁸¹

Since this question has been studied with special care by Polayes and Derby,⁸² the results of their experiments will be cited in some detail. As is shown in table 92 the average incubation period in the group of patients receiving compatible blood is 4.30 ± 0.22 days, while in the group receiving incompatible blood it is 8.18 ± 0.48 days. The differ

⁷⁸ *Genetics* 25: 18 (1940) cf. Wiener *Human Biology* 7: 222 (1935)

⁷⁹ Cf. Rife *Ohio Jour Sci* 38: 281 (1938)

⁸⁰ *Wien klin Woch* 40: 345 (1927) 41: 1304 (1928)

⁸¹ *Wettmar Klin Woch* 6: 1947 (1927) *Deutsch med Woch* 54: 826 (1928)
Hopf *Munch med Woch* 75: 1755 (1928) etc.

⁸² Polayes and Derby *Jour Amer Med Assoc* 102: 1126 (1934) Derby *Amer Jour Psych* 91: 881 (1935)

TABLE 92

INCUBATION PERIOD OF INJECTION MALARIA IN PATIENTS RECEIVING COMPATIBLE AND INCOMPATIBLE BLOOD
(After Polayes and Derby)

Incubation Period (Days)	Patients Receiving Compatible Blood		Patients Receiving Incompatible Blood	
	Number of Cases	Per Cent	Number of Cases	Per Cent
1	8	10.1	2	4.8
2	7	8.8	0	0
3	22	27.7	2	4.8
4	15	18.9	3	7.1
5	13	16.4	4	9.5
6	5	6.3	5	11.9
7	4	5.0	7	16.7
8	0	0	5	11.9
9	1	1.3	3	7.1
10	0	0	2	4.8
11	0	0	1	2.4
12	1	1.3	2	4.8
13	1	1.3	2	4.8
14	0	0	1	2.4
15	1	1.3	0	0
16	0	0	0	0
17	0	0	1	2.4
18-19	0	0	0	0
20	1	1.3	0	0
21-22	0	0	0	0
23	0	0	2	4.8
Totals	79	100.0	42	100.0

ence between the mean incubation periods of the two groups is 3.88 ± 0.52 days and is therefore certainly significant.

To be sure there is also a wide variation in the incubation periods within each group but this is probably due to such factors as individual resistance to malarial infection variations in the stage of the cycle when the blood was taken variations in the quantity of blood injected etc. Of these individual resistance is probably most important. Thus O'Leary and Ashton could not induce chills or fever in 10 per cent of 984 patients they injected and in the series reported by Polayes and Derby there were 6 cases (3 in each group) in which malaria could not be induced. These cases were not included in the calculations.

Several theories have been suggested to explain the prolongation of the incubation period of injection malaria when incompatible blood is used. The theory that seems most plausible is that the hemolysis of the

injected blood cells sets free the malarial parasites, which then cannot develop properly until they have found new hosts. Furthermore on the basis of *in vitro* experiments, Horn²³ believes that in the process of agglutination and hemolysis of the red blood corpuscles, many of the parasites are killed or injured.

²³ *Wien klin Woch* 30: 995 (1929)

CHAPTER XXI

MEDICOLEGAL APPLICATION OF BLOOD TESTS IN DISPUTED PARENTAGE

SINCE THE agglutinogens A, B, M, and N of human blood are inherited in accordance with definite laws, blood grouping can be applied in medicolegal cases where the problem of determining blood relationship arises. Examples of cases in which blood grouping may prove of value are the following:

1 A child is born in lawful wedlock and the husband denies paternity. The law presumes that all children born in lawful wedlock are legitimate (in order to protect the interests of the child) unless it is proved that it is impossible for the husband to be the father of the child. As proof of non paternity the courts permit the introduction of evidence of non access, and of impotence or sterility of the husband.

2 A child is born out of lawful wedlock, and the man named by the mother as its father denies paternity.

3 Two newborn infants have been accidentally interchanged in a hospital shortly after birth, and it is desired to identify the parents of the infants. Or it is suspected that a wet nurse has wilfully substituted her own infant for the one placed in her custody, so that her own child may have the benefits of a better home.

4 A woman has simulated pregnancy and child birth and now pretends that a certain child is her own, in order to compel a man to marry her, or to obtain dower in her dead husband's estate (in jurisdictions where birth of issue is a prerequisite to the right of dower), or in order that the child may become the heir to her husband's estate.

As has already been pointed out, the mechanism of inheritance of the four blood groups may be summarized in the following two laws:

1 The agglutinogens A and B cannot appear in the blood of a child unless present in the blood of one or both parents.

2 A parent belonging to group AB cannot give rise to a group O child and a group O parent cannot give rise to a group AB child.

What the groups of the children must be, when the groups of the parents are known, is shown in table 93.

In order to illustrate the application of blood grouping in affiliation cases, a case which was solved by this method will be cited.

In this case, an unmarried woman accused a certain man of the paternity of her child. It was found that the woman belonged to group O, the child to group B and the putative father to group A. Since the child possessed agglutinin B which was

TABLE 93

THE LANDSTEINER BLOOD GROUPS IN PARENTS AND CHILDREN

Groups of Parents	Groups of Children Possible	Groups of Children not Possible
1 O×O	O	A B AB
2 O×A	O A	B, AB
3 O×B	O, B	A AB
4 A×A	O A	B AB
5 A×B	O A B, AB	—
6 B×B	O B	A, AB
7 O×AB	A, B	O AB
8 A×AB	A B, AB	O
9 B×AB	A B, AB	O
10 AB×AB	A, B, AB	O

not present in the mother's blood this agglutinin must have been derived from the father who could therefore only belong either to group B or group AB. When confronted with this evidence the woman withdrew her charge and the falsely accused man was released.

Suppose, however, that in the case just cited it had been found that the putative father belonged to group B or group AB. This would not prove that the accused man was the actual father of the child any more than any other man belonging to one of these two groups. Obviously, blood grouping is of value only for excluding paternity, and not for proving paternity.

The two laws cited above are derived from the Bernstein theory of heredity of the blood groups. The great majority of investigators apply this theory without reservation. However, on account of certain apparent exceptions to the theory, slight objections have been raised to placing exclusions of parentage based on the second law on the same level as those made under the first law of heredity. In the preceding edition of this book it was therefore recommended that nonconformity to the first law be considered absolute proof of non parentage, while nonconformity to the second law should perhaps only be considered evidence that parentage is extremely unlikely.¹ Since more recent studies on the heredity of the blood groups have served to remove all reasonable doubt concerning the accuracy of the Bernstein theory, in the author's opinion this slight reservation with regard to the second law of heredity is hardly necessary any longer (cf. Chapter XI). This

¹ It is of interest to note that approximately five sixths of the exclusions possible are made by the first law, whereas only one sixth of the exclusions fall under the second law.

statement is made even conceding that the case reported by Haselhorst and Lauer may be well established (cf page 185), because this exception stands alone among the tens of thousands of examinations that have been made

It may be of interest to point out that the exception reported by Haselhorst and Lauer involves a child (born June 16 1928) who is a deafmute nearly completely blind and has the left side of the face deformed.² These pathological findings have led some authors to question whether this can be considered a valid exception to the rules of heredity in *normal* individuals rather than some abnormality resulting from a chromosomal aberration.³

TABLE 94

CHANCES OF PROVING NON-PATERNITY WITH THE BLOOD GROUPS
(Where putative father has been falsely accused)

	Frequencies of Groups				Per Cent Exclusions Putative Father in Group†				
	O	A	B	AB	O	A	B	AB	Unknown
European (and American)	39	43	12	6	23.5	7.7	14.6	39.9	16.3
Maximum *	31.3	29.6	29.6	9.7	29.0	13.4	13.4	31.1	20.0

* This is a theoretical population giving maximum chances of excluding paternity

† Cf. Hooker and Boyd *Jour Immunol* 16: 451 (1929) Wiener et al *Jour Immunol* 19: 259 (1930)

It is unfortunate that the reservation with regard to the second law expressed in the previous edition of this book has been used as an excuse by the Domestic Relations Court in New York City⁴ for adjudging a man of group AB to be the father of a child belonging to group O. The type of ecclesiastical reasoning used by the court in arriving at its decision can be seen from the following quotation: "Feminine dignity throughout all generations and races eternally alert and vigilant to resent the infamy of such a charge (that her child is illegitimate) and outraged at being thus degraded by a test of its decency and morality by a court order (for a blood test) issued without any evidence in the first instance to sustain it never forgives or forgets this deadly insult." As is pointed out by Schatkin⁵ this line of reasoning does not cancel the *concrete objective* evidence of non-paternity supplied by the blood test. It is significant that in the present case the wife admitted antenuptial relations with her husband who was induced to marry her when informed of her pregnant condition.

² Haselhorst and Lauer *Ztschr Konstitutionslehre* 15: 205 (1930) 16: 227 (1931)

³ Cf. Schiff and Boyd *Blood Grouping Technic* p. 135 (1942)

⁴ *Harding vs Harding* N.Y. Supp. (2d) 22: 810 (1940)

⁵ *Jour of Crim. Law and Criminology* 32: 458 (1941)

As not in every case where the putative father has been falsely accused will blood grouping establish his innocence, it becomes interesting to determine the exact chances of proving non-paternity in such cases. This will vary with the blood group of the individual and to a certain extent with the distribution of the blood groups in the population. As may be seen in table 94, the average chance of establishing non-paternity for most races ranges between 16 and 19 per cent (approximately 1 in 6, or more). Europeans of group A, however, have only 1

TABLE 95

MEDICOLEGAL APPLICATION OF BLOOD GROUPING IN EUROPE (UP TO 1932)
(After Levine*)

Country	Number of Cases	Paternity Exclusions	Percentage Exclusions
Germany (Schiff)	4519	353	7.8
Austria (Werkgartner)	700	63	9.0
Danzig (Puschet)	600	39	6.5
Denmark (Thomsen)	50	6	12.0
Denmark (Sand)	500	64	12.8
Sweden (Wolff)	259	17	6.6
Norway	37	4	10.8
Switzerland			
Lithuania			
Total	6665	546	8.2

* *Amer Jour Police Science* 3: 157 (1932) [Modified after Schiff, *Med Welt* No 34 (1929)]

chance in 13 of establishing their innocence, while the most favorable group is group AB, with the chances equal to 2 in 5.*

Although the average chance of proving non-paternity by means of the blood groups is 1 in 6, of course in practice fewer exclusions will be made, since many of the men accused are the actual fathers. In table 95 are listed the results of blood grouping tests in 6655 medicolegal cases. The average percentage of exclusions is 8.2 per cent, whereas, as is shown in table 94, approximately 16.3 per cent of exclusions would be expected if all the men were falsely accused. This would indicate therefore, that half of the defendants in these cases were falsely accused,

* General formulae for the chances of proving non-paternity in terms of the frequencies of the three genes A, B, and O, have been derived independently by Wiener [*Jour Immunol* 19: 259 (1930); 24: 443 (1933)] Koller [*Ztschr f Rassenphysiol* 3: 121 (1931)] and Zarnick [*Med Pregled* 5: 1 (1930)]. The chances of establishing non-paternity are given by the formula,

$$P = p(q+r)^4 + q(p+r)^4 + pqr^2(p+q) + 2pqr^2.$$

so that only half were the true fathers.⁷ The procedure of permitting the woman to name the father of her child therefore must lead to considerable injustice by making many men support children not their own. Routine blood grouping tests in such cases will not only establish the innocence of a large number of these men but also will serve to diminish the number of false accusations, because of the complainant's fear that she may be charged with perjury.

Occasionally it may only be possible to obtain the bloods of the man and child, but not of the mother. For example, a man who suspects his wife of infidelity may desire to obtain further evidence before making any charges. In certain cases, as when the man and child both belong to group A, it is useless to go further, since irrespective of the group of the mother, non paternity cannot be established (cf table 93). On the other hand, if the man belongs to group AB and the child to group O or vice versa non paternity is proved, regardless what group the mother belongs to (cf page 380). The chances of proving non paternity when only the bloods of man and child are available are naturally very small⁸ and for European peoples approximately equal to 1 in 20 (4.7 per cent). In other cases, e.g. when the man belongs to group O and the child to group A the group of the mother must be determined before it is possible to make a decision.

It has already been pointed out that blood grouping can only be used to exclude but not to prove paternity. Occasionally, however, blood grouping may aid in determining in an indirect way who the true father is. If there is evidence that one of two men must be the father and one of them is excluded by the blood grouping tests it follows that the other is the father of the child.⁹ In Germany, the exclusion was of the greatest importance to the child, for in that country if the plea of *exceptio plurium concubentium* was raised the natural child had no right at all unless it could be proved that only one of the men could possibly be the father.

Unfortunately no serologic method is known which differentiates reliably homozygous and heterozygous individuals of group A and group B. If such a method were established the chances of proving non paternity by means of the blood groups would be increased by approximately 10 per cent.

From an examination of the blood of his parents it may be possible in certain cases to determine whether an individual of group A (or B) is homozygous or

⁷ Schiff *Lancet* p 921 (1929).

⁸ Namely equal to twice the product of the frequencies of group O and group AB [$2\bar{O} \times \bar{AB}$].

⁹ That too much reliance should not be placed on the testimony of the mother in such cases is emphasized by the observation of instances in which both of the putative fathers have been excluded by the blood tests [See Schiff *Deutsche Zeitschr f d ges gerichtl Med* 18 14 (1931)].

TABLE 96

PROTOCOL ILLUSTRATING EXAMINATION OF BLOODS IN A MEDICOLEGAL CASE

Cell Suspension of	Serum of			Test Sera of				Blood Group
	L R	E N	R N	Group A		Group B		
				1	2	1	2	
L R (Putative father)	-	++±	++	-	-	++±	++±	A O B
E N (Mother)	-	-	-	-	-	-	-	
R N (Child)	++±	++±	-	++±	++	-	-	
Controls *								
Group O	-	-	-	-	-	-	-	
Group A ₁	-	++±	++	-	-	++±	++±	
Group A ₂	-	++	+	-	-	++±	++	
Group B	++±	++	-	++±	++	-	-	

* Controls of both subgroups A₁ and A₂ are necessary in order to avoid errors such as those mentioned on page 35

heterozygous. For instance, if an individual belongs to group A and only one of his parents possesses agglutinin A, he is heterozygous. On the other hand, in the rare mating AB × AB, all the group A and group B children must be homozygous.

As has already been pointed out, blood grouping is also valuable in cases where infants have been accidentally interchanged in a hospital. A solution is possible in more than 40 per cent of such cases.¹⁰ As an illustration, a case that occurred in a hospital in Chicago during the summer of 1930 will be cited. This case, which received a good deal of newspaper publicity,¹¹ was finally solved by means of blood grouping tests.

After Mrs. H. had returned home from the hospital, it was found that her baby had a label on its back with the name 'B'. The baby at the B house was found to bear a label with name 'H'. When the bloods of the six individuals involved were examined, the following results were obtained:

	Group		Group
Mr. B	AB	Mr. H	O
Mrs. B	O	Mrs. H	O
Baby labelled 'H'	O	Baby labelled 'B'	A

Since Mr. B belongs to group AB, he cannot have a child of group O, so that the child in his custody could not be his, whereas the child labelled 'B' belonging to group A could. On the other hand, since Mr. and Mrs. H. both belonged to group O, the child in their custody could not be theirs. It is obvious, therefore, that the babies

¹⁰ Wiener Ztschr. f. indukt. Abstamm. u. Vererbungslehre 59: 227 (1931); Jour. Immunol. 24: 443 (1933).

¹¹ A complete description of this case may be found in the magazine *Liberty* for October 11, 1930, page 36.

had been properly labelled but had been accidentally interchanged before the mothers went home.

In the hands of qualified serologists, any of the various methods of blood grouping described in Chapter II will be found suitable for medico-legal cases. It is imperative, however, that both cells and serum of each individual be examined. Furthermore, adequate control tests in sufficient number should be carried out. As a further check on the technique and as a check on the labelling, especially where the blood has been received by mail, the present writer makes a practice of performing the tests on two independent sets of samples of blood. A sample protocol taken from the medico-legal case described on page 380 is shown in table 96.

Schiff has pointed out that if the sera of both the mother and father agglu-

TABLE 97

THE AGGLUTINOGENS M AND N IN PARENTS AND CHILDREN

Types of Parents	Types of Children Possible	Types of Children Not Possible
1 MN × MN	M, N, and MN	—
2 MN × N	N and MN	M
3 MN × M	M and MN	N
4 M × N	MN	M and N
5 N × N	N	M and MN
6 M × M	M	N and MN

minate the cells of the child, non paternity is established (cf. table 96) except for the case where the parents belong to group A and group B, respectively, and the child to group AB. The exceptional case mentioned is readily recognized without the aid of grouping tests by cross matching the bloods of the two parents since mutual agglutination should then occur. This direct test has the possible advantage that the results may be clearer to the layman, like cross matching tests prior to transfusion.

A question frequently asked is how old the child must be before its blood can be tested. The blood group can always be determined at birth; although the isoagglutinins are not fully developed until the child is several months to a year old, the group can be determined from the reactions of the cells (cf. page 25). In rare cases where the results are not clean-cut, however, it may be necessary to repeat the tests when the child is older and the agglutinins have appeared in the serum. The author has made it a practice in affiliation cases not to carry out the tests until the child is at least one month of age.

Application of the Agglutinogens M and N in Affiliation Cases. The mechanism of heredity of the agglutinogens M and N was discussed in Chapter XIV. It was pointed out in that chapter that all the available

TABLE 98

CHANCES OF PROVING NON PATERNITY WITH THE AGGLUTINOGENS M AND N

Racial Type	Frequencies of Types			Per Cent Exclusions Putative Father in Type			
	M	N	MN	M	N	MN	Un known
European	29.2	21.2	49.6	34.6	40.6	0	18.6
Maximum*	25.0	25.0	50.0	37.5	37.5	0	18.75

* This is a theoretical population giving the maximum chances of proving non paternity

evidence, namely, studies on families containing a total of more than 15 thousand children and individual examinations of over one hundred thousand bloods fully support the theory of heredity proposed by Landsteiner and Levine so that its medicolegal application is amply justified at present. Therefore in all cases where a decision is not possible from the tests for A and B tests for M and N should be performed.

The relationship between the MN types of the children and the types of the parents is shown in table 97.

As is shown in table 98, a man falsely accused of paternity has approximately 1 chance in 6 (18.6 per cent) of proving his innocence by this method.¹¹ Men belonging to type MN have no chance whereas men of type M or N have more than 1 chance in 3 of proving their innocence.

If the agglutinogens M and N as well as A B are used therefore the

TABLE 99

CHANCES OF PROVING NON PATERNITY WITH THE AGGLUTINOGENS A B M AND N*

Group	O			A			B			AB			Un known
	M	N	MN	M	N	MN	M	N	MN	M	N	MN	
Chances (Per Cent)	50.0	54.6	23.5	39.6	45.1	17.7	44.1	49.3	14.6	60.7	63.4	39.9	31.9

* Based on the frequencies of the blood types in New York City

average chances of proving non paternity are doubled so that 1 out of 3 falsely accused men can now be exonerated. If the blood groups and

¹¹ If m and n represent the frequencies of genes M and N respectively then the average chance of proving non paternity by means of the agglutinogens M and N is given by the formula (Wiener Schiff Koller)

$$P = mn (1 mn)$$

MN-type of the falsely accused man are both known, the probabilities of establishing his innocence are as shown in table 99

The medicolegal application of the agglutinogens M and N depends upon the following two laws, previously formulated

1. If the child possesses an agglutininogen M or N not present in the blood of one or both parents, non-paternity is established. One-third of the exclusions possible belong to this class

2. If either of the combinations, type M man with type N child or type N man with type M child are found, non-paternity is established.¹¹ This type of exclusion is of particular importance, because at times it is difficult or impossible to obtain the mother's blood (cf page 384). Since two-thirds of the MN exclusions are of this type, these factors are of special value in such instances

As regards interchange of infants, a solution is possible in more than 40 per cent of the cases, by means of the agglutinogens M and N alone. If all four agglutinogens A, B, M, and N are used, almost 70 per cent of such cases can be completely solved.¹²

The present author has had the opportunity to apply the agglutinogens M and N in many instances of disputed paternity. One of these, which is particularly interesting, will be described here.

After eight years of married life, during which time she had had frequent intercourse with her husband, but had failed to become pregnant, Mrs. X met and fell in love with Mr. Y. During the ensuing five years three children were born. In the meantime, the persons involved had tried to come to an understanding and wished to determine which of the two men was the father of each child. The bloods of those involved were examined with the following results:

Blood of	Group	Type
Husband	O	MN
Lover	A	N
Wife	O	MN
First child	O	MN
Second child	O	M
Third child	A	N

It is obvious that the husband could not have been the father of the third child (belonging to group A), since both he and his wife belong to group O. On the other hand, since a type N man cannot give rise to a type M child, the lover could not be the father of the second child. By means of these tests, therefore, it has been shown indirectly that the husband is the father of the second child and the lover the father of the third child. No decision can be rendered concerning the first child.

¹¹ Theoretically, it would also be possible, in a small percentage of cases, to prove non-paternity even from the bloods of the supposed paternal grandparents. Thus from grandparents M×M grandchildren of type N cannot result and similarly grandparents N×N cannot have a type M grandchild. (Schiff *loc cit*.)

¹² Wiener, *Ztschr f indukt Abstamm u Vererbungs* 59: 227 (1931), *Jour Immunol* 24: 443 (1933).

The technic to be used when testing for M and N has already been described (cf page 219) Since these tests offer more difficulty than the tests for A and B, they should only be intrusted to experts, i e, workers who have had considerable experience with the technic The reciprocal relationship between isoagglutinin and isoagglutininogen, which serves as such a valuable check when testing for A and B, does not obtain here, of course It is of the utmost importance, therefore, to perform the tests with several different testing fluids of each kind, and to include a sufficient number of control bloods in each experiment

The main difficulty in the technic lies in the danger of false negative reactions for N, on account of the occurrence of bloods which give only weak reactions¹⁵ In medicolegal cases one must show that the blood in question is completely negative, even on microscopic examination, although other N bloods of low sensitivity, especially heterozygous blood, give distinct reactions with the same reagents Naturally the investigator will offer an opinion only when the results are unmistakable

Blood Grouping in Disputed Maternity Blood tests can also be applied in the relatively rare instances of disputed maternity Maternity is disproved in any of the following four combinations (1) Putative mother—group AB, child—group O, (2) putative mother—group O, child—group AB, (3) putative mother—type M, child—type N, (4) putative mother—type N, child—type M The chance of proving non maternity with the aid of the classic blood groups alone is relatively small (on account of the rare occurrence of group AB), and for European races approximately equal to 1 in 20¹⁶ On the other hand, the tests for M N are much more useful in cases of this nature since the chance of proving non maternity with the latter tests is higher,¹⁷ namely, for European countries approximately equal to 1 in 8 Hirszfeld¹⁸ has reported a number of cases in which he was able to prove non maternity with the aid of the agglutinogens A and B A case recently seen by the author and which was solved by the tests for M and N is of interest

In 1935 Mrs T a woman in Buffalo N Y brought an action against her husband for separation and he counterclaimed for annulment of the marriage on the ground of fraud The facts were that up to the time of the trial the woman had been in the state of matrimony seven times During her sixth venture she had had an affair with her present husband and the child in question was supposed to be the fruit of that clandestine relationship Mr T however denied that the child was his he asserted in fact that it was not even his wife's insisting that he had been inveigled into the marriage on the pretext that it was. A blood test was

¹⁵ With regard to the rare type MN₂ cf page 227

¹⁶ Equal to twice the product of the frequencies of groups O and AB

¹⁷ Equal to twice the product of the frequencies of types M and N

¹⁸ *Ztschr f d ges gerichtl Med* 27 70 (1936)

ordered by the court and Dr S L Vaughan found that Mr T belonged to group B the alleged mother to group A and the child to group A so that as far as the A B groups were concerned no definite conclusion could be drawn The bloods were then forwarded to the author for M N tests and it was found that Mrs T belonged to type N and the child to type M so that she could not be the mother of the child It may be added that evidence was presented at the trial to show that the plaintiff had had her uterus and tubes removed by an operation performed long before the child was born Mrs T explained the abdominal scar by asserting that she had had her child by Caesarean section although she could not produce the surgeon who was supposed to have performed the operation However the husband who preceded Mr T testified that the plaintiff had not been pregnant at the time the child was born that she had in fact never menstruated as long as he had known her Finally it was learned that Mrs T had obtained the child from an orphan asylum where it had been placed after the death of its mother

Application of Other Serologic Characters in Disputed Parentage When blood examinations are carried out for court cases, tests for the A B groups and M N types are now routine If the appropriate reagents are available it may also prove worth while to carry out tests for the subgroups of groups A and AB and factors P, Rh and S These additional tests have certain disadvantages, however Firstly, the number of investigations on the heredity of these properties is still relatively small, so that the results of the tests can be accepted only with reservation Secondly, the four additional tests combined raise the chances of excluding paternity from about 33 to only about 40 per cent the contribution of each test being merely 2 to 4 per cent

With regard to the subgroups of groups A and AB investigations during the past few years have served to substantiate the theory of Thomsen, Friedenreich and Worsaae (cf Chapter XII) When tests for the subgroups are made, paternity can be excluded under the following two rules (1) No child can belong to subgroup A₁ or A B unless one or both parents belongs to one of these subgroups (2) A B parents cannot have A₂ children and A₂ parents cannot have A₁B children The tests are carried out most simply and satisfactorily with the aid of highly active absorbed B sera (cf page 199) Since the blood of new born infants usually gives weak reactions with such reagents more reliable results will be obtained if the subgrouping tests are carried out after the infant is at least one or two months old Definite contradictions to the rules of heredity of the subgroups should not be placed on the same level as exclusions by means of the A B groups or M N types, though they render the likelihood of parentage remote If the results are not clean cut and if any of the bloods give intermediate reactions (cf page 218), no opinion should be rendered

In the heredity studies on the secretor type thus far carried out no exceptions have been encountered to the rule that no child can be a secretor unless one or both parents are secretors (cf table 61) Ac

cording to Christiaens,¹⁹ the characters S and s were applied for the exclusion of paternity by Popielski in Poland before the war. The present author has carried out this test in a number of paternity cases, but prefers not to use it when any of the individuals involved belongs to group O. The reasons for this are firstly that suitable antisera for testing group O saliva are difficult to obtain, and secondly that the distinction between secretors and non secretors is not as sharp in this group as in groups A, B and AB.

The property P has been used by Dahr and Zehner²⁰ in cases of disputed parentage in Germany. In a series of 21 cases they succeeded in establishing nonpaternity 3 times with the aid of this test. In the first case, the woman withdrew her accusation when confronted with the result of the test, and in the second case the man was also excluded by the A B and M N tests (putative father OMP, mother OMP, child A₁MNP). The main obstacle to the general application of property P in cases of disputed parentage is the lack of a regular source of potent antisera (cf. page 256).

With regard to the Rh tests, in only about 2 per cent of the cases (complainant and putative father both Rh negative) is there a possibility of excluding paternity. In addition more investigations on the heredity of the Rh factor are desirable (cf. page 250). However, the present author has made Rh tests in paternity proceedings for the past two years and has already had one interesting result.²¹

In this case before the Court of Special Sessions of New York City an unmarried woman charged a certain man with the paternity of her child. The blood grouping tests gave the following results:

Blood of	Group	Subgroup	Type	Rh Reaction
Putative father	B		M	Negative
Mother	AB	A ₂ B	M	Negative
Child	AB	A ₁ B	MN	Positive

Accordingly the results of three independent tests, namely for the subgroups MN types and Rh factor, all served to exclude the accused man. Confronted with this evidence the complainant, who had previously denied contact with anyone but the putative father, now recalled a second man, but stated her contact with him had occurred at a time which made it impossible for him to be the father. This story of course failed to impress the court, which acquitted the defendant as soon as the results of the blood tests were made known to it.

Procedure in Paternity Cases. In medicolegal cases it is important to properly identify all the individuals involved or the results of the

¹⁹ *La Recherche de la Paternité par les Groupes Sanguins*. Masson et Cie. Paris (1939).

²⁰ *Deutsche med. Woch.* 67, 11 (1941).

²¹ Landsteiner and Wiener. *Jour. Exp. Med.* 74, 309 (1941).

passed by the New York State Legislature in 1935 and 1936, giving courts in that state power to compel individuals involved in paternity disputes to submit to blood grouping tests. Shortly thereafter similar laws were passed in Wisconsin,³¹ and, more recently, in Ohio, Maine, New Jersey, South Dakota and Maryland. The Committee on Medico-legal Blood Grouping Tests of the American Medical Association has expressed the opinion that such laws should be passed in all states in this country.³² A few years ago in New South Wales, Australia laws were passed giving courts the power to order blood tests in cases where parentage is in issue.³³ Similar laws were proposed in the House of Lords in England,³⁴ where they were favorably received but not enacted into the law on account of the war. Thomas³⁵ has drafted a model law, which appears to combine the best features of the New York and Wisconsin laws and is therefore cited here as a model.

Whenever it is relevant in any action to determine the parentage or identity of any person or body the Court may direct any party to the action and child of any such party and any other person involved to submit to one or more blood grouping tests to determine whether the defendant can be excluded as being the father of the child and the specimens for the purpose to be taken and the tests made by one or more registered medical practitioners³⁶ as the Court shall direct who are specially qualified by training and experience in the making of blood group classifications and under such directions as the Court shall deem proper.

Whenever such tests are ordered and made the results thereof shall be admissible as evidence but only in those cases in which exclusion is established.

The order for such blood grouping tests may also with the consent of all parties direct that a certified copy of the evidence of such experts may be admissible as evidence without the attendance of such experts at Court. The Court shall determine how and by whom the costs of such examinations shall be paid.

Whenever the Court orders such blood grouping tests to be made and one or more of the parties shall refuse to submit to such tests such fact shall be disclosed to the Court unless good cause is shown to the contrary.

Up to 1930 only the classic blood grouping tests were applied in medicolegal cases. Since 1930 the tests for M N have been included in the routine blood examination in cases of disputed paternity. In table 100 are listed data collected from the literature and with the aid of questionnaires, summarizing the results of blood grouping and M N tests in various European countries³⁷ and in the United States. By com-

³¹ *Laws Wisconsin* 1935 chapter 351

³² *Jour Amer Med Assoc* 108 2133 2215 (1937)

³³ *Jour Amer Med Assoc* 113 159 (1939)

³⁴ *Brit Med Jour* 2 1210 (1938)

³⁵ *Jour Crim Law* 1 598 (1937)

³⁶ To this the present writer would add the phrase "or other well qualified individuals" in order not to exclude geneticists and other scientists who have made a special study of the subject.

³⁷ Data beyond 1937 were not available for Europe because of the war.

paring this table with table 95 it will be seen that the newer tests for M N have doubled the percentage of exclusions obtained

Table 100 is by no means complete since there is undoubtedly a good deal of unpublished data which has not come to the author's attention. The data concerning Austria may be supplemented by the following information supplied by Dr Franz Josef Holzer. Blood grouping was first introduced into the courts of Austria in cases of disputed parentage as early as 1920 and the M N tests were introduced in 1932. Since 1930 the blood tests have been used in almost every paternity case at least in Vienna and there the number of tests done every year numbers many thousands. In Graz during the period 1920-1936 there were 1250 paternity cases in which the blood tests for A and B were made in 418 of which M N typings were also performed and in Innsbruck 40 to 50 tests were made every year.

In Denmark the tests were most extensively applied probably because of the comprehensive research work done in that country. The data given in table 100 can be supplemented by the following information taken from Christiaens obtained in response to a questionnaire.

At the Medicolegal Institute of the University of Copenhagen approximately 7500 tests have been made over a period of 12 years. During the years 1933 to 1936 the results in 3124 cases were

Number of Cases	Exclusions					Exclusions Not Possible
	A B O	M N	A ₁ A ₂	A B O and M N	A ₁ A ₂ and M N	
3 124	256	376	52	81	10	2 349

During the first year that the M N tests were used many old cases were reopened in which tests previously made for A B alone had been inconclusive. This accounts for the higher frequency of exclusions with the M N tests.

It may be of interest to note that in certain countries the blood grouping tests are rarely applied no provision having been made for them by law. In Italy for example paternity may not be disputed except in cases of rape. Leone Lattes the most prominent authority on the subject in that country states that his experience includes only a few private cases and he does not know of any case before the Italian courts in which the M N tests have been used.

Thomas points out that the number of affiliation applications in England and Wales averages 7200 each year, and an average of more than 2000 of the defendants are sent to prison every year for non-payment. Thomas urges the routine use of the blood tests in all these cases since he estimates that at least 1100 men could be proved innocent in England by these tests each year.

In the United States, as is shown in table 100, the blood tests have been most widely used in New York State³⁵ on account of the laws

³⁵ In addition to New York State the tests have thus far been used in this country in California New Jersey Connecticut Massachusetts Illinois Ohio Maryland Pennsylvania and Wisconsin. Other states are being added to this list.

TABLE 100

AVAILABLE REPORTS ON THE USE OF BLOOD GROUPING AND M N TESTS IN EUROPEAN (1930-1937) AND IN ENGLISH AND AMERICAN COURTS (1932-1942)

Investigator	Country	Number of Cases	Number of Exclusions			Per Cent Exclusions
			By Tests for A & B	By Tests for M & N	Totals*	
Schiff, <i>Deutsche Ztschr f d ges gerichtl Med</i> 21 404 (1933)	Germany	911	69	82	139	14.7
Dambrowsky, <i>Med Belt</i> 1938 832	Germany	691	46	38	76	12.5
Wolff and Jonsson, <i>Acta Pathol et Microbiol Scand</i> 12 131 (1935)	Sweden	600	36	38	69	11.5
Clausen, <i>Ztschr Rassenphysiol</i> 4 420 (1934)	Denmark	119	12	15	25	21.0
Routil, <i>Mitt d Anthropol Gesellsch in Wien</i> 65 233 (1935)	Austria	129	11	10	21	16.3
Püschel, <i>Ztschr f Immunol</i> 81 445 (1934)	Danzig	156	17	17	32	20.5
Hirsfeld, L. personal communication	Poland	180†	9	7	13	7.2
Friedenreich, V. cited after Thomas, <i>Jour Crim Law</i> 1 598 (1937)	Denmark	1778			425†	23.9
Harley and Roche Lynch, <i>Lancet</i> 1 911 (1940)	England	50	4	5	8	16.0
Wiener, <i>U S Law Review</i> 70 683 (1935) and unpublished data	USA (mostly N Y C)	432	27	24	46	10.7
Levine <i>N J Law Jour</i> Oct 5 (1939)	USA (N J and N Y)	81	1	6	7	8.7
Witek, <i>Proc N Y State Assoc of Publ Health Labs</i> 20 9 (1940) and unpublished data	USA (Buffalo, N Y)	131	8	11	18	13.7
Guttmacher, personal communication	USA (Baltimore, Md)	74	7	3	10	13.5
Evans, personal communication	USA (Cal)	133†	8	6	14	10.5
Hyman and Snyder, <i>Ohio State Law Jour</i> 12 203 (1936), Hyman, unpublished	USA (Ohio)	75	3	10	13	17.3

* Figures in this column are less than the sum of the two preceding columns since, naturally, there are cases of exclusions made by both methods

† Tests for M and N not done in a few cases

h were passed there. However, even in New York State the tests only being used in a small fraction of the cases of disputed paternity. In New York City alone an average of over 1000 proceedings are held every year. One factor probably responsible for this is the of familiarity of the legal profession with the blood tests. This situation is gradually being corrected as a result of the publication of number of good reviews of the subject in law journals.³⁹ Another important factor is the cost of the examination. However, the expense becomes insignificant when it is realized that if the defendant is convicted he must contribute towards the support of the child until the child is 16 years of age.

Once the blood grouping tests have been made and an exclusion obtained the results are almost invariably accepted by the courts as valid. However, in rare instances, as in two cases in this country, it may be possible for a clever prosecuting attorney so to confound the jury with extraneous pseudo evidence that he may obtain a conviction in the face of scientific proof excluding paternity.

In one case (reported by Heise⁴⁰) a man was adjudged to be the father even though blood grouping tests excluded him because of a fancied resemblance between the accused man's father and the infant. The judge found the decision to be against the evidence and granted a new trial. It is significant that the woman never appealed with the new trial for the reason that although both she and the putative father were white the child's features gradually became negroid as it grew older.

In the second case a children's court in California decided against a man 70 years old and impotent despite the fact that the blood tests exonerated him. This erroneous verdict was reversed in an excellent decision rendered by the appellate court. Unfortunately however on further appeal to the Supreme Court the verdict of the trial court was upheld on purely legal technical grounds.⁴¹

To the skeptic the most convincing demonstration of the accuracy of blood tests is the behavior of the woman when confronted with the results of the tests. In the

Britt *Minn Law Review* 21 671 (1937) Muehlberger and Inbau *Jour Crim Law and Criminol* 27 578 (1936) Wiener *St John's Law Review* 8 70 (1933) *U S Law Review* 70 683 (1936) Flacks *Amer Bar Assoc Jour* 21 (1935) *ibid* 23 4 2 (1937) Koch *St John's Law Review* 9 102 (1934) Ker and Boyd *Jour Crim Law and Criminol* 25 187 (1934) Vogelhuber *Portland Law Rev* 6 101 (1936) Galton *Oregon Law Review* 17 177 (1938) *more Evidence* (3d ed 1940) 165a b 2220 etc.

Amer Jour Clin Path 4 400 (1934) This case is cited as *Commonwealth v Amarelli* 17 Pa D & C 229.

Arais v Kalensikoff (Cal) 67 P (2d) 1009 Rev 74 P (2d) 1043 Ab cited in *Jour Amer Med Assoc* 110 466 (1938) Cf Britt *Minn Law Review* 836 (1938) Jennings *Cal Law Rev* 26 456 (1938) Other unfavorable decisions were made by other courts as follows *State v Holad* 24 N E 2d 962 *Marik v Bednarik* 16 Atl 2d 810 *Harding v Harding* 22 N Y Supp 2d 80 These decisions are criticized in the following law reviews *Jour Crim Law and Criminol* 31 523 (1939) *Bill of Rights Rev* 1 226 (1940) *St John's Law Rev* 228 (1940).

vast majority of cases the woman readily admits the falsity of her accusation when informed that the putative father has been excluded. Naturally there are some exceptions and the one cited by Judge Wylegala is most instructive.⁴² The girl involved lived next to a fire house and her story was well corroborated that one of the firemen a married man had taken her out several times a week over a period of more than two years. The fireman's defense was weak and his alibi highly improbable. When he demanded a blood test his attorney advised him to save his money. Surprisingly the test excluded him. The girl stuck to her story and the tests were repeated with the same result. When questioned again the girl now recalled how one night she accepted a ride from an unknown man who forced himself on her. She insisted however that he could not possibly be the father since she had been with him only once.

Several courts in the United States have rendered rather exhaustive and learned opinions favoring the blood tests.⁴³ It is to be expected that as the legal profession acquires greater familiarity with the blood grouping tests and their usefulness in cases of disputed paternity they will be applied more and more frequently. Particularly it is hoped that in those jurisdictions where statutory authority is necessary to enable courts to order these tests suitable legislation will be passed in accordance with the law proposed above.

It seems hardly necessary to repeat that the testimony only of fully qualified experts should be accepted. Reports of rather gross mistakes obviously due to insufficient familiarity with the serologic and genetic aspects of the subject can be found in the literature.⁴⁴

⁴² Witebsky and Wylegala *Blood Grouping and Typing Tests in Affiliation Cases* (Papers read at 19th Annual Conference of Judges of Children's Courts of New York State Auburn N.Y. Oct. 3, 1941, privately printed).

⁴³ *Matter of Swahn* 285 N.Y. Supp. 234; *State v. Damm* 266 N.W. 667, 104 A.L.R. 430; *State v. Helling* 6 Ohio Opinions 371; *State v. Wright* 59 Ohio Appeals 191 and 17 N.E. 2nd 428; *Beach v. Beach* 114 F. (2d) 479 (1940); *Euclide v. State* 286 N.W. 3; *Schulz v. Schulz* 35 N.Y. Supp. 2d 218; *Anderson v. Anderson* (Ill.) N.Y. Law Jour. 108, 603 (1942).

⁴⁴ Lauer *Med. Welt* 8, 933 (1934); Report of Committee on Medicolegal Blood Grouping Tests *Jour. Amer. Med. Assoc.* 108, 2133 (1937); Wiener *Isda Nuova* 42, 131 (1938); Wiener *Jour. Amer. Med. Assoc.* 117, 216 (1941).

CHAPTER XXII

INDIVIDUAL IDENTIFICATION OF STAINS IN FORENSIC CASES

Blood grouping has proved of value not only in disputed parentage but also in criminal cases where it is of importance to determine the origin of blood stains. This application was first suggested by Landsteiner and Richter,¹ who showed that it is possible to demonstrate the presence of isoagglutinins in dried blood stains. Grouping tests on blood stains were first applied in criminal cases by Lattes,² in 1916, in a proceeding before an Italian criminal court. From that date onward, the number of cases in which the method was used gradually increased, and the tests were adopted by courts in several other countries, particularly in Germany,³ Japan,⁴ France,⁵ England,⁶ U S A, etc. The following case may serve to illustrate the principles underlying the application of grouping of blood stains.⁷

A woman's corpse was discovered which had stab wounds on its face. Two country lads who possessed a metal implement were apprehended. The sharp edges of this implement which corresponded in size and shape to the wounds on the murdered woman's face were covered with brown stains. Nevertheless the youths denied any connection with the crime. Examination of the stains on the weapon showed that they consisted of group B blood whereas the murdered woman belonged to group O. Several days before the trial, the real murderers were found.

As is shown in the case just cited, blood grouping can be used to prove that a given blood stain could not have come from a certain individual, namely, when the blood stain is of a group different from that of the person from whom it is supposedly derived. On the other hand, the fact that the blood stain is of the same group as that of a given individual is of course no proof that the blood came from that

¹ 74th meeting of *Deutsche Naturforschung und Aert e* Karlsbad (1902) *Ztschr f Medizinalbeamte* 16 85 (1903)

² *Arch de Anthropol crim e Med leg* 37 422 and 538 (1916)

³ Goroncy abstracts in *Deutsche Ztschr f d ges gerichtl Med* 11 409 (1928), 13 234 (1929), Strassmann *Deutsche Ztschr f d ges gerichtl Med* 5 184 (1925) etc

⁴ Fujiwara *Deutsche Ztschr f d ges gerichtl Med* 15 470 (1930)

⁵ Martin and Rochaix *Ann Med leg* 5 1 (1925) Dujarric de la Riviere and Kossovitch *ibid* 7 390 (1927)

⁶ Editorial *British Med Jour* (March 5, 1932), *New York Law Jour* (Feb 13 1932)

⁷ Popoff *Ukrain Zentrabl Blutgruppenforsch* 3 177 (1929)

person. Nevertheless, such information may be of value as confirmatory circumstantial evidence.*

A murder had been committed by stabbing. From the nature of the wound it seemed certain that some of the blood must have spurted on the murderer's clothes. Upon adequate grounds, suspicion of the murder centered upon a man K. He, however, denied any knowledge of it. The clothes of K were secured one month after the crime, and on them several brown irregular stains were found which by chemical and spectroscopic tests proved to be blood. The precipitin test showed that it was human blood, and further examination revealed that the blood belonged to group B, which was the same as that of the murdered individual. On the other hand, K was found to belong to group A. K then confessed his crime.

Hence, blood grouping tests may help not only to acquit the innocent but also to convict the guilty. As an additional illustration, the following is given. Suppose a criminal is wounded in a chase by the police but makes good his escape. Some of his blood left at the scene of the crime is found to belong to group A, type N. Then all suspects except those belonging to this group and type can be eliminated and much time may be saved. As a result, the likelihood of apprehending the real criminal is correspondingly increased. Therefore, it may eventually be found to be desirable to keep records not only of every criminal's fingerprints but also of his blood group. Incidentally, although it may be possible to destroy fingerprints of criminals by a surgical operation, it is not possible to change their blood groups.

As mentioned above, if in murder cases, grouping tests reveal that the blood found on the suspect's clothing is not, as he claims, his own, this can constitute the most important link in the chain of evidence. When tests are made for only the four classic blood groups the chance that the accused man and the victim belong to different groups is $1 - (\overline{O}^2 + \overline{A}^2 + \overline{B}^2 + \overline{AB}^2)$. For most white races this would be approximately $1 - (0.40^2 + 0.40^2 + 0.15^2 + 0.05^2)$, or about 65.5 per cent.[†] That is, in about two thirds of the cases of this sort the blood grouping tests may prove of value, the chances varying according to the group of the defendant. If tests are also made for the MN types and the subgroups of group A and AB, the chance that the bloods of the suspect and victim can be distinguished from one another are increased to almost 90 per cent. It is obvious, therefore that when examining blood stains, the tests should be made as complete as possible.

If the blood is still fresh when found, so that blood suspensions can be prepared, the tests for all the groups and types can be done in the usual manner. With dried blood stains a much more difficult technic is required, and the investigator is restricted to the determination of the four blood groups and the presence or absence of M, since with dried blood the tests for the property N and the subgroups of groups A and AB are not yet reliable. For best results with blood grouping tests in

* Fujiwara, *loc cit*

[†] This chance is considerably greater than that of paternity exclusion (cf. page 382)

criminal cases, therefore, police officers should be instructed that all blood stains found at the scene of the crime are immediately to be brought to the laboratory for examination. Since the determination of the group becomes more and more difficult with the passage of time after death, it is advisable that the determination of the group and M N type should be made a routine part of the post mortem examination in all cases of death by violence, whether or not there is suspicion of murder at the time.

Inasmuch as properties corresponding to the agglutinogens A and B can be demonstrated in the seminal fluid (cf Chapter XVII), grouping tests may also prove useful in cases of rape or assault (cf page 416). With regard to such stains, the same general principles apply as for blood but here the tests are limited to the determination of the four groups alone (cf page 294).

TECHNIC OF EXAMINING BLOOD STAINS

When examining a supposed blood stain it is first necessary to establish that the stain actually contains blood. This can be done by the usual chemical methods. Spectroscopic examination may be of value.¹⁰ After it has been proved that the stain really consists of blood it is necessary to determine whether or not the blood is of human origin. If the blood is sufficiently well preserved a microscopic examination should be performed since the morphology of the cells may give an indication of their origin (E g, if the cells are nucleated and elliptical they are not of mammalian origin). To establish the human origin of the blood however, precipitin tests must be performed.¹¹ For convenience, a brief outline of this test will be given here.

Precipitin Test

Preparation of Precipitin Sera. The precipitating immune sera are obtained most readily by injecting rabbits with human serum. Satisfactory results can be obtained by giving courses of 6 or 7 daily intravenous injections of small doses of serum (e g 2 c.c. of a 1:10 dilution) alternating with rest periods of 7 to 10 days. The first injection of the 2nd and later courses may be given subcutaneously. The rabbits are bled one week after the last injection of a course. Some rabbits produce potent sera after 2 courses of injections others only after 3 or more courses. At any rate the rabbits should be bled as soon as the titer is sufficiently high since if the injec-

¹⁰ For a full description of the various chemical and spectroscopic tests for blood see Gonzales Vance and Helpert *Legal Medicine and Toxicology* Chapter XXIII D. Appleton Century Co. N.Y. (1937).

Cf Uhlenhuth and Seiffert *Biologische Erweisendifferenzierung mittels der Precipitation mit besonderer Berücksichtigung der Technik*. *Kolle Wassermann's Handbuch der Pathogenen Mikroorganismen* III 1 365-468 (1930). Smith and Glaister *Recent Advances in Forensic Medicine* Chapters VI and VII Philadelphia (1939).

tions are prolonged the specificity (and potency) of the serum may decrease. When antisera must be obtained in a short time one can follow the recommendation to give 3 intraperitoneal injections on successive days of 5 cc 10 cc and 15 cc. of serum respectively, and bleed the rabbits on the ninth day.

To be satisfactory the immune sera must be perfectly clear sterile of high potency and of high specificity. The sera will usually be clear if the rabbits are given no food for 18 hours before the bleeding. To secure sterility the sera can be filtered through a Berkefeld or Seitz filter though if the rabbits are bled aseptically this step is not essential. As a preservative 0.5 per cent phenol and other chemicals have been suggested, the writer uses 1:10 000 methiolate. The serum need not be inactivated. Kept in the refrigerator with or without the addition of a preservative precipitating sera are often usable even after years. A usual method

TABLE 101

SCHEME FOR PRECIPITIN TEST (Alt & Hektoen*)

-
- | | |
|---|---|
| 1 | Extract of blood stain+antiserum |
| 2 | Extract of blood stain+normal rabbit serum (Estimated dilution of blood stain in Tubes 1 and 2, 1:1000) |
| 3 | Extract of bloodless part of shirt+antiserum |
| 4 | Salt solution+antiserum |
| 5 | Human blood or serum 1:1000+antiserum |
| 6 | Blood or serum other than human 1:1000+antiserum |
-

* *Jour Amer Med Assoc* 70 1273 (1918)

for determining the titer of an immune serum is to test it against dilutions of human serum as follows. Into a series of small test tubes 0.5 cc. of 1:100 1:1000 1:5000 1:10 000 and 1:20 000 dilutions (in normal saline) of human serum are placed. Then 0.1 cc. portions of the antiserum to be titrated are added. With a satisfactory precipitating serum clouding is evident in the first two tubes at once at the latest in 1 to 2 minutes. After a longer time turbidity may be apparent at a dilution of antigen of 1:10 000 or higher. A good immune serum is not only of high potency but also specific, thus, in tests made with 1:100 and 1:500 dilutions of serum from an unrelated species no turbidity should be visible even after 20 minutes.

Preparation of Extracts The actual test is performed on a 1:1000 dilution of the stain in normal saline solution. Since in practice most stains will be dry and often not completely soluble this can only be approximate. The proper dilution can be recognized in the following ways: the solution appears clear and almost colorless when held up to the light, a foam that tends to persist forms if the solution is blown through with a pipette, and a slight cloudiness appears on addition of nitric acid or on heating. If there is any doubt two or more different dilutions should be prepared and all tested. Old stains are usually more difficult to extract than fresh stains and if the extraction requires more than two hours it should be carried out in the refrigerator. Turbid extracts must be clarified by filtration or centrifugation. The reaction of the extract is tested with litmus paper. As a rule the reaction will be neutral otherwise the solution should be neutralized before making the test. As a control extracts are made of unstained portions of the material from which the blood stain is taken particularly of clothing which may have been in contact with organic matter capable of reacting with the precipitin sera.

The Tests The precipitin test for a blood stain on a shirt for example can be conducted as shown in table 101

In each tube is placed 0.2 to 0.5 c.c. of extract or control solution and 0.1 c.c. of anti human rabbit serum or normal rabbit serum (cf table 101) is added. After mixing the tubes are watched for the appearance of a grayish white turbidity or precipitate. If a precipitate is formed within a few minutes in tubes 1 and 5 but no precipitate appears in any of the other tubes after 20 minutes it may be concluded that the blood is of human (or monkey) origin. The technic of the tests can be varied e.g. with 5 drops of extract or control solution 1 drop of serum is mixed and the mixture examined for turbidity. In forensic cases the tests should be done in duplicate preferably with two different immune sera.

The author prefers to carry out precipitin tests routinely in narrow tubes by the ring method. This technic gives sharp results and is at the same time economical of material. The tubes are prepared from narrow glass tubing (2 mm inside diameter) by cutting it into 5 inch lengths and sealing one end of each piece of tubing in a Bunsen flame. Instead of sealing the tubes with a flame one can stick them vertically into a tray filled with plasticine in order to close the bottom ends. In the tests the precipitin serum is first placed with the aid of a capillary pipette into the bottom of the tube so as to form a column about one-quarter inch high then the extract is carefully pipetted above it care being taken not to trap any air bubbles between the two layers. The precipitate is easier to see if the tubes are held up to the light and a dark background held slightly below the level of the liquid is interposed between the light and the tube.

By reason of the high sensitivity of the precipitin test one must be careful that all glassware and pipettes used have been thoroughly cleaned. Individual pipettes must be used for every specimen and reagent and all reagents particularly the salt solution must be free from contamination.

Heating alcohol formaldehyde and various other chemicals interfere with the precipitin test. However blood stains 15 to 60 years old have been successfully identified. In fact it is stated that positive precipitin reactions have been obtained with the extracts of muscle tissue of Egyptian and American mummies. Since dried blood resists deterioration much better than fluid a few samples should be dried on filter paper or glass and preserved in that state whenever the examinations cannot be carried out at once.

If the precipitin reaction is positive this does not necessarily prove that the stain consists of human blood since other material of human origin for example semen muscle extracts etc. react with anti human precipitin sera. To prove that the stain actually consists of blood chemical or spectroscopic tests must be done as mentioned above.

Human blood stains can also be identified with the aid of precipitin sera against human hemoglobin.¹² For a number of years the present author has routinely carried out this test alongside with the usual chemical and precipitin tests in homicide cases. The main disadvantage of this excellent test is that the antisera are difficult to produce because hemoglobin is a relatively weak antigen. A satisfactory hemoglobin solution for immunizing rabbits and for use as a control can be prepared simply as follows. The erythrocytes of a citrated blood sample are washed several times with saline to remove the plasma and to the packed cells 9 volumes of distilled water are added in order to hemolyse them. The solution is made isotonic by adding one-tenth volume of 10 per cent salt solution and then cleared by centrifugation or passing it through a Seitz filter. In a few cases the author

¹² Hekteen and Schulhof *Jour Inf Dis* 33 224 (1923)

has obtained positive results with the anti hemoglobin serum where the usual precipitin serum failed

If the precipitin reaction is negative this means either that the stain is not of human origin or that the specimen has deteriorated. It is desirable to have on hand a variety of precipitating immune sera for blood of domestic animals such as cattle sheep dogs chickens etc.¹³ since if the test with anti human serum is negative tests with one of the other anti sera might be positive and this would solve the problem. With stains containing mixtures of bloods from two species tests with both of the respective precipitating sera will be positive.

Group Reactions In an extensive study of precipitins for blood sera from numerous animal species involving in all about 16 000 tests Nuttall¹⁴ observed that precipitins usually reacted not only with the serum injected (homologous or specific reaction) but also with the sera of closely related species (group reaction).¹⁵ This applies not only to precipitins for serum but also for hemoglobin so that as pointed out positive results in the precipitin reaction as ordinarily carried out prove the presence of human blood only if monkey blood can be excluded. While usually the circumstances preclude this possibility it may in very rare cases be necessary to distinguish between human and monkey blood. Should a distinction between human and monkey blood be necessary an extremely improbable case the differentiation could easily be made with a properly absorbed serum (cf below).

Precipitin Reaction of Seminal Fluid Seminal stains can frequently be identified by simple microscopic examination of stained preparations since the sperm cells usually resist drying. Hektoen¹⁶ has recommended the use of precipitin sera for identifying such stains particularly where the sperm cells are no longer recognizable. The injection into rabbits of animal or human seminal fluid induces the formation of specific precipitins which however in most cases also precipitate the homologous blood serum. Hektoen states that the precipitins for blood serum in anti semen serum can usually be removed completely by treating the precipitin serum with an equal volume of a 1:200 dilution of the corresponding serum. Since the removal of cross reactions from precipitin sera also has other applications the method will be given here in some detail.

The simplest method of eliminating cross-reactions is to use diluted (e.g. 1:2 or 1:4) instead of concentrated antiserum in the test because sufficiently diluted antiserum will usually precipitate only the homologous antigen. Better results may perhaps be obtained by absorbing the antiserum with the heterologous antigen so as to remove the group precipitins. According to Schiff and Boyd² the antiserum should be treated with that quantity of heterologous antigen with which it reacts most rapidly (optimal proportions of Dean and Webb). To determine this tests are made with the diluted antiserum (e.g. 1:5 or 1:10) against a series of dilutions of the heterologous antigen and the rates of flocculation are compared. The quantity in the tube showing precipitation most promptly is multiplied by

¹³ If anti rabbit immune sera are needed these are prepared by immunizing chickens.

¹⁴ Nuttall *Blood Immunity and Blood Relationships—The Precipitin Test for Blood* 214 pages (1904).

¹⁵ Occasional precipitin sera were encountered which reacted not only with sera from related species but also with low dilutions of mammalian sera in general (mammalian reaction). Sera of this type are of course unsatisfactory for forensic work.

¹⁶ Hektoen and Rukinstat *Arch Path* 6:96 (1928).

¹⁷ *Blood Grouping Technic* p. 163 (1942).

5 or 10, as the case may be, to obtain the appropriate amount to be added to the antiserum. The proper amount of antigen is added to the antiserum taking care that the volume of the former is less than half the volume of the latter, and the mixture is allowed to stand overnight in the refrigerator. The precipitate is removed by centrifugation, and, as a rule, the supernatant will now be found to react with only slightly diminished potency with the homologous antigen while the group reaction no longer occurs. If the group reaction has not been completely removed the absorption should be repeated with a smaller quantity of the heterologous antigen.

Grouping Tests

As has already been mentioned, if the blood stains are still wet when found, all that is necessary is to drop a small amount of the stain into saline solution, and to test the resulting suspension like fresh blood. If a large enough clot is present, the serum should be separated and examined for its isoagglutinin content.

While carrying out routine grouping tests on postmortem blood samples in homicide cases, the present author had an interesting experience. One blood sample gave reactions corresponding to group A but the cells failed to agglutinate in either anti M or anti N serum. Suspicion was aroused by this result, especially since the erythrocytes appeared slightly smaller than usual, the bottle was not labelled and the amount of blood supplied (4 ounces) was extraordinarily large considering the age of the deceased (6 months). Moreover, tests on the serum showed that it agglutinated all human bloods. Precipitin tests were then carried out, and negative reactions were obtained with anti-human precipitins, positive reactions with an anti-ox precipitin serum that was available at the time. Inquiry revealed that the messenger in his haste had picked up a bottle of sheep blood instead of the blood sample of the deceased.

Since, as already mentioned, grouping tests on dried blood stains are much more difficult and at the same time apt to be less informative than tests on wet stains, wet stains that cannot be examined promptly should be carefully preserved in order to prevent drying, hemolysis and bacterial contamination. Police officers¹⁸ finding such stains at a distance from the laboratory should drop them into a bottle or tube which is tightly stoppered and packed in ice (not dry ice!), taking care to avoid any direct contact between the stain and the ice. A small portion of the stain may also be placed in a tube containing a small amount of isotonic salt solution (readily prepared by dissolving about two heaping teaspoonsful of table salt in a quart of water). If drying cannot be prevented, as in the case of thin stains on knife blades, the instrument should not be packed until drying is complete, or the stain may be taken up by the wrapping paper. Needless to say each article should be packed individually, especially when the stains are wet.

As Schiff and Boyd¹⁹ state, the grouping of dried blood stains, though

¹⁸ Cf. Holzer, *Ergeb d ges Med* 20. 368 (1935)

¹⁹ *Blood Grouping Technique*, p 158 (1942)

simple in principle, is much more delicate and exacting than other serological tests such as the Wassermann test, especially since an accused man's life might conceivably depend on the results (cf page 420). These authors point out that individuals familiar with other aspects of blood grouping are not necessarily equipped to carry out such tests and advise that no one should venture to carry out these examinations in medicolegal cases unless he has previously had experience in serology and correctly grouped a large number of stains.

When examining dried blood stains one must plan the tests so as to utilize the material available to the best possible advantage. Where the stains are large, no special plan of approach is necessary while with very small stains one may have to forego the grouping tests entirely and limit oneself to the chemical and precipitin tests. As is indicated below, stains on hard surfaces and on adsorbent materials are grouped by different technics, so that each case should be individualized. Where the amount of stain is limited, considerable experience is required in order to extract the maximum possible information.

Wherever possible, dried blood stains should be examined for their content of both isoagglutinins and isoagglutinogens. Since the isoagglutinins are more labile than the isoagglutinogens the tests for the latter are more apt to succeed. However, the isoagglutinins can also be detected in dried blood after a considerable time²⁰ and inasmuch as the methods of demonstrating isoagglutinins are simpler (and were developed earlier) they will be described first.

Technic of Testing for Isoagglutinins in Dried Blood Stains When testing stains for their isoagglutinin content, one must have available fresh blood suspensions of known group and of high sensitivity, preferably washed once with saline solution. Since different group B bloods do not vary significantly in sensitivity, any sample of fresh group B blood is suitable, for group A test cells an individual of subgroup A₁ should be selected, in addition, group O blood should be available for use as a control.

Tests for isoagglutinins can be carried out either directly on the dried blood stain itself or on extracts of the stain. The direct test is particularly suitable when the blood stain is in the form of a crust, for example, for stains on non absorbent materials such as glass, metal, stone, etc. Surprisingly, suitable crusts can be obtained at times from garments, particularly when the blood has been sprayed on the surface.

²⁰ Levine was able to detect both isoagglutinins α and β in a dried blood stain 4 years old [*Amer Jour Police Sci* 3: 157 (1932)]. Also see Balgares and Christaen *Ann de Méd Lég* 17: 215 (1937). The latter investigators could demonstrate the presence of the isoagglutinins in almost all their dried blood stains kept for 9 months and in more than half their stains that had been exposed to sunlight during this period.

in the form of droplets which dry quickly before they can soak into the material. Best results are obtained with small thin crusts, under ideal conditions positive results may be obtained with quantities as

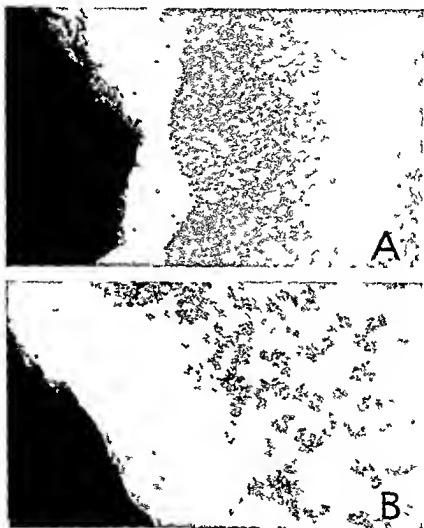


FIG 68 TESTS FOR AGGLUTININS IN A DRIED BLOOD STAIN
(Magnified 90X)

A Negative reaction B Positive reaction The blood crust can be seen on the left and the test red blood corpuscles on the right. Between the crust and red blood cells is a clear zone that forms as the dried blood goes into solution.

small as 1 or 2 milligrams. Paradoxically, thicker crusts give less satisfactory results, possibly because larger stains dry more slowly, allowing time for the blood clot to retract and express part of the serum.

Equal portions of the blood crusts are placed on each of three glass slides, and to these are added one drop of freshly prepared 2 per cent blood suspensions of the standard O, A₁ and B bloods, respectively. A cover slip is immediately dropped over each mixture, and if crusts of suitable thickness have been selected, the cover slip will lie flat and the drop of blood suspension will spread until it fills the space between the cover glass and slide. The slides are placed in a moist chamber for about 30 minutes, being removed during this interval from time to time for examination under the low power lens of the microscope. With thicker blood crusts it may be necessary to press gently on the cover slip in order to make it lie flat and to help the blood stain dissolve. In positive reactions, at first only the cells immediately adjacent to the blood crust are clumped, since there the concentration of agglutinins is highest. With the passage of time more and more of the isoagglutinins in the stain are dissolved and at times complete agglutination may result. The microscopic appearance of typical positive and negative reactions is shown in figure 68. Gentle pressure on the coverslip should be applied while reading the reactions under the microscope, since this will have little or no effect on true isoagglutination but will usually cause rouleaux formation to break up. In addition, the tests against group O cells should show no trace of agglutination.

For blood stains on cloth or similar absorbent materials it has been recommended to extract with minimal amounts of distilled water and then to evaporate the resulting red solution obtained to dryness on glass at room temperature. The powder obtained in this way (or by scraping the original stain off a hard surface) is mixed with very small quantities of distilled water to make a paste, and this is permitted to dry on a glass slide (or coverslip). To the dry crusts which result the test cell suspensions are added.

On the whole, the reactions with these artificial blood crusts are not as satisfactory as those obtained with natural crusts. Somewhat better results may at times be achieved with the aid of extracts of the blood stain. This method is particularly suitable for stains on cloth provided that sufficient material is available for examination. A portion of the stained cloth is thoroughly moistened with a small quantity of distilled water or weak saline solution. It may be necessary to manipulate and squeeze the cloth with the aid of a forceps or a clamp. After about 5 to 10 minutes (with old blood stains the period of extraction required may be much longer) as much of the liquid as possible is expressed from the cloth, and in this way a dark red extract of the stain is obtained. If the blood is in the form of powder it may be extracted in the cold with 4 times its weight of weak saline solution. After a few hours the mixture is centrifuged and the supernatant fluid taken

off The extract is then tested like ordinary serum against standard cell suspensions of groups O, A₁ and B

Better results can sometimes be obtained by using larger amounts of extract and weaker blood cell suspensions in order to increase the sensitivity of the tests (cf page 20) Five drops of blood stain extract, clarified by centrifugation, are placed in each of 3 small test tubes and one drop is added of freshly prepared 0.5 per cent suspensions of O, A₁ and B blood, respectively The mixtures are allowed to stand for 5 to 10 minutes and then centrifuged at low speed for 1 minute The supernatant dark fluid is decanted and to the sedimented cells one drop of saline is added The tubes are then gently shaken and the reactions read

In interpreting the results of tests for the presence of isoagglutinins in dried blood stains it must be borne in mind that only positive findings are of significance Agglutination of either or both suspensions, A₁ and B, proves that the corresponding isoagglutinin is present in the stain Absence of agglutination does not permit the conclusion that the isoagglutinins were originally absent, as the agglutinins may have deteriorated If the standard group A and B cells are both agglutinated but the group O cells are not, the stain must belong to group O However, if only the A cells are agglutinated and not B, for example, the stain may belong to group B, but it could also belong to group O if the isoagglutinin β has deteriorated, as well could happen particularly if it was weak to begin with In any event, such a stain could not belong to group A or group AB

When testing for isoagglutinins, pseudoagglutination must be carefully guarded against The differentiation between isoagglutination and pseudoagglutination has already been discussed (cf page 36) The addition of lecithin to the cell suspension has been recommended for the prevention of pseudoagglutination (Lattes), but the writer has never found it necessary to resort to the use of this device, since the criteria described in table 10 for recognizing and preventing pseudoagglutination were sufficient

Although pseudoagglutination is the most frequent cause of error when examining blood stains, the other possible sources of error must also be considered (cf Chapter III)

Landsteiner and Richter showed that it is possible to prove that a blood stain could not have come from a given individual without first determining the group of the stain Thus, if an extract of the blood stain is found to agglutinate the red blood cells of the individual, the stain could not have been derived from that person's blood It is always best, however, to combine this method with the actual determination of the blood group of the stain, the two tests serving as mutual checks

Technic of Testing for Isoagglutinogens in Blood Stains The presence

of agglutinogens in dried blood stains is determined by the capacity of the stain to specifically absorb agglutinins so that the technic is similar in principle to that employed for demonstrating group specific substances in tissues and secretion (cf Chapter XVII)

The grouping of blood stains by the absorption technic has certain limitations and pitfalls which the investigator must constantly bear in mind. Prolonged drying of blood stains denatures them and eventually renders them largely insoluble under which conditions they lose their capacity to absorb the group agglutinins while most secretions containing group substances tend to remain soluble. Accordingly the failure of a blood stain to absorb agglutinins is not conclusive evidence of the absence of the corresponding agglutininogen. Since many bloodstained articles especially underwear in practice have proved to contain group specific substances this not infrequently interferes with determination of the agglutininogen content of a blood stain. While freshly laundered clothing give no group reactions after being worn a few days they are likely to give reactions corresponding to the blood group of the owner and even apparently clean nonabsorbent objects such as doorknobs furnace shakers etc. which are handled a good deal may give group specific reactions. Accordingly the serologist should never undertake the grouping of blood stains by the absorption technic without testing simultaneously apparently unstained portions of the object. Where the bloodstained article is too large to be sent to the laboratory the police officer should remove the blood stain together with a liberal portion of the unstained area. If an unstained control is not available the absorption test should not be attempted and the examination should be limited merely to tests for the agglutinin content.

In selecting testing sera for the absorption tests the same general principles apply as those presented on page 280 when discussing the examination of organs and secretions for group substances. Obviously more material is needed to appreciably reduce the titer of a potent serum than to completely absorb a weaker serum on the other hand with too weak a serum a slight nonspecific reduction in titer may simulate a specific reaction. Reagents with a limiting titer of 8 to 16 are satisfactory and as was pointed out on page 284 better results are obtained by using potent sera diluted with saline than by using undiluted low titered sera. If there is only enough blood stain for a single absorption equal parts of double strength anti A and anti B sera should be mixed and this pooled serum used for the tests. This is preferable to the use of group O serum since the agglutinins present in such serum are often of unequal titer, also when group O sera are treated with either A or B blood there may be a distinct diminution in the titer of the heterologous agglutinin (cf page 26) ²¹

²¹ In this connect on see the paper by Harley [*Brit Jour Exp Path* 17 33]

In testing for the presence or absence of agglutinin A, cognizance must be taken of the fact that the A agglutinogens in the two sub groups A_1 and A_2 , and in group AB, differ not only in their sensitivity to agglutination, but also in their capacity to absorb anti A agglutinins

TABLE 102

ABSORPTION TESTS OF TWO "STRONG" B SERA WITH BLOOD STAINS OF TYPES A_1 A_1B , A_2 A_2B AND O (After Therkelsen)

Serum No	Absorbed with Stain	Test Cell Suspension	1 1	1 2	1 4	1 8	1 16	1 32	1 64	1 128	1 256
2 Serum H. H. Jensen Diluted 1 : 8	A	A	-								
	A	A ₁	-								
	AB	A	+	(+)	-						
	A	A	-								
	A ₂	A	++	++	+	(+)	-				
	A	A ₁	-								
	AB	A	++	++	++	+	(+)	-			
	A	A	(+)	-							
	O	A	+++	++	++	+	+	(+)	-		
	A	A ₁	+++	++	++	+	(+)				
	A	A ₂	+++	+++	++	+	+	(+)	-		
	Control 1 : 8 Untreated Serum	A	++	++	+	((+))	((+))				
	A	A	+++	+++	++	++	++	+	(+)	-	
	A	A	+++	+++	+++	++	(+)	-			
6 Serum S. Jørgensen Diluted 1 : 8	A	A	+	+	-						
	A ₁	A ₁	-								
	AB	A	++	+	+	(+)	-				
	A	A	+	-							
	A	A	++	++	++	+	(+)	-			
	A ₁	A	+	-							
	AB	A	+++	+++	++	++	++	+	+	(+)	-
	A	A ₁	+++	++	++	++	+	+	(+)	-	
	O	A	+++	+++	+++	++	++	+	+	+	(+)
	A	A	+++	+++	+++	++	++	+	(+)	-	
	A	A	+++	+++	+++	++	++	++	++	(+)	-
	Control 1 : 8 Untreated Serum	A	+++	+++	++	+	+	(+)	-		
	A	A	+++	+++	+++	++	++	++	+	+	(+)
	A	A	+++	+++	+++	++	++	+	+	(+)	-

The control used in this experiment was of linen which had been thoroughly laundered and boiled three times in distilled water. In the titrations, the figures given (1 : 1, 1 : 2, etc.) represent further dilutions of the 8 times diluted sera.

This is illustrated very well in table 102, which is taken from a study by Therkelsen.²² Incidentally, this table also demonstrates that if the sera used in the absorption tests are too strong, it may be impossible to demonstrate the presence of weak agglutinogens, such as the agglutinin A in A_2B blood. The tests for B are carried out in a similar manner using standard A serum and test cells B (the question of sub

(1936)] who encountered an unusual group O serum exhibiting the property that while blood of group B removed only the anti B agglutinin blood of group A absorbed the isoagglutinins not only for A but also for B blood

²² *Ztschr f Rassenphysiol* 8 : 98 (1936)

groups not playing a part here) The titrations and tests can be carried out either in small test tubes or on slides, the former being preferable (cf page 13)

If the stain is found to be in the form of a crust, this is reduced to a fine powder This is important because in this manner the surface of the stain in contact with the serum will be increased and the absorption will be more complete Small amounts of powder are added separately to group A and group B serum, respectively, in two small test tubes in the proportion of 10 mg to every 0.1 c.c. of standard serum (titer about 16) After the mixtures have stood for 30 to 60 minutes at room temperature, the tubes are centrifuged and the supernatant fluid tested for its agglutinin content The absence of the isoagglutinin from either test serum or a significant reduction in the titer of the test serum indicates the presence of the corresponding agglutininogen in the blood stain According to Schiff, absorption tests may be supplemented by applying the method of splitting off agglutinins (cf page 27)

Where stains are spread over non absorbent surfaces in a thin layer the following technic has been used by the author with success Two or three drops of the testing serum are placed on the stain, and these are sucked back and forth with a capillary pipette until the stain is completely dissolved or suspended If the amount of stain taken up is not enough to turn the serum dark brown the serum is dropped on a fresh portion of the blood stain and the process repeated The mixture is then placed in a narrow tube (2 mm inside diameter) and allowed to stand in the refrigerator (As a control a few drops of testing serum are treated at the same time and in the same way with an unstained portion of the surface of the article) The following day the tubes are centrifuged and the supernatant serum tested for its agglutinin content For the group A testing serum, any fresh group B suspension may be used for the group B testing serum, best results will be obtained if blood suspensions of subgroup A₁ are selected (cf page 279)

For stains on cloth or other similar absorbent material the author has found the following technic to be satisfactory Equal portions of the stained cloth are placed on a watch glass To each is added only as much standard serum of group A and group B (titer 8-16) as the stained cloth will hold It is often helpful to manipulate and press the hard, dry stained cloth so that it will absorb the serum Small pieces of unstained cloth are treated in a similar manner After 10 to 15 minutes, each piece of cloth is pressed in order to squeeze out the serum as completely as possible If the tests have been carried out properly and the stain is not too old, the serum recovered from the stained cloth will be dark red in color Each portion of serum obtained is then tested against known cells of groups A₁, A₂ and B The reactions obtained by this method in an actual medicolegal case are shown in figure 69 The

A serum
treated
with
stained
cloth

B serum
treated
with
stained
cloth

A serum
treated
with
unstained
cloth

B serum
treated
with
unstained
cloth

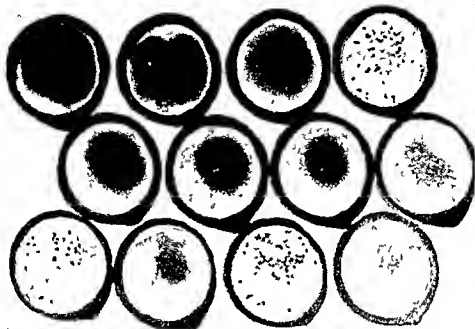


FIG 69 DETERMINATION OF THE GROUP OF A DRIED BLOOD STAIN ON AN OVERCOAT (1½ diameters)

The tests have been set up on a paraffin ring slide. In each cell of the first row was placed one small drop (.025 cc) of A_1 cells, in each cell of the 2nd row, A_2 cells, in each cell in the 3rd row, B cells. To the first cell of each row was added a small drop of A serum that had been treated with the blood stained portions of the cloth, to the 2nd cell of each row, B serum treated with the blood stain, to the 3rd cell of each row, A serum treated with unstained cloth, to the 4th cell of each row, B serum treated with unstained cloth. The stain absorbed agglutinin α but not β and therefore belongs to group A.

method is most successful if the blood-stained material is relatively thick, so that the stain is compact. When the blood is spread thinly over a wide surface, the agglutinins may be incompletely absorbed, even though the stain appears to be large. It should be emphasized again that the control test with the unstained cloth must never be omitted; if possible, the control portions of unstained cloth should be taken from the immediate vicinity of the blood stain itself.

The dark brown color of the solutions recovered after absorption may interfere with the reading of the agglutination tests. The reactions are more easily read if the tests are made on slides and the layer of fluid is thin, or the tests can be conducted by the technic suggested by Clausen

(cf page 416). In a way, the color of the solutions offers an advantage by indicating the amount of dissolved material and the solubility of the stain. If, for instance, the color is very light, there is not much chance of success.

If there is very little material for testing, the technic described by Boyd and Boyd²² may be found suitable. An amount of stained material containing only about 4 to 5 mg of dried blood (equivalent to about $\frac{1}{3}$ of a drop of blood) is taken. If the blood stain is found on linen, for example this quantity would be spread over 1 square centimeter of the material. Two such samples are placed in two small test tubes to which are added 0.1 cc of A and B sera, respectively (diluted so that their limiting titers are 8 to 16). The blood stain and serum in each tube are prodded with the aid of a thin glass rod so that the material becomes wet. The mixtures are placed in the ice box for 24 hours after which time some of the fluid is removed and tested. A second test is made after an additional 24 hours. The tests are set up by the hanging drop method on cover slips sealed with vaseline on to well slides. The amount of serum and material is so chosen that there is barely sufficient excess of serum to yield a few loopfuls for testing. When it is difficult to recover the fluid, a little can usually be drawn up through a capillary pipette pressed against the material.

Boyd and Boyd, in making tests for isoagglutinogens in blood stains have also obtained satisfactory results by using standard sera prepared from properly diluted and absorbed immune rabbit sera against A and B (cf page 31). If sufficient material is available the use of such reagents in addition to the usual standard group A and group B sera constitutes a check on the findings.

It must be pointed out that, when interpreting the results of the absorption test for isoagglutinogens in blood stains, just as with the tests for isoagglutinins, only positive findings are of significance. Failure to demonstrate any isoagglutinogens does not necessarily mean that the blood stain belongs to group O, because the agglutinogens originally present may have been destroyed, or the amount of material used for the test may have been inadequate or the technic of the test not sufficiently sensitive. However, with proper technic, the failure of a blood stain to absorb the isoagglutinins is a valuable check on the successful demonstration of the presence of both isoagglutinins anti-A and anti-B in the stain, and is practically diagnostic alone if the material is fresh and sufficient in amount. If the stain is found to absorb anti-B agglutinins but not anti-A, the stain cannot belong to group O or group A, but it is not possible to assert with certainty whether it belongs to group B or to subgroup A_2B . This may then be decided by testing the stain for its agglutinin content. If the stain (shown not to contain any agglutinins) absorbs both agglutinins anti-A and anti-B, because of the possibility of non-specific absorption, the diagnosis AB may still be open to question. This diagnosis is supported if control tests show that heteroagglutinins for animal blood, e g, rabbit blood, present in the test sera are not absorbed.

²² *Jour Immunol.* 33: 159 (1937).

Attempts to identify group O as a positive property, which would be particularly valuable for stains whose agglutinins have deteriorated, have not yet completely succeeded. As shown in table 103, the use of anti-O sera may make this possible, but suitable reagents are difficult to obtain and, as a rule, the reactions are not clean-cut.

TABLE 103

DEMONSTRATION OF THE PROPERTY O IN GROUP O BLOOD STAINS

The reagent used for these tests was prepared by absorbing a particular normal or serum, found to contain anti O (or anti A₂) agglutinins, with packed washed human red blood cells or subgroup A₁. 0.1 c.c. portions of the reagent were treated with 10 mg. of each pulverized dry blood stain. The reagent was tested before and after absorption against O, A₂, and A₁ cells. Note that the group O stains not only absorb the anti O agglutinins, but also give off anti A agglutinins.

Anti O (α ₂) Serum Treated With Blood Stain of	Tested Against Cell Suspension		
	O	A ₂	A ₁
J C Group O	f tr	tr	+ ±
J S Group O	tr	+ ±	++
D McL Group O	tr	tr	+
J St Group A ₁	+ ±	+ ±	-
B W Group A ₁	±	±	-
Wm F Group B	+ ±	+ ±	+ ±
Control—Untreated Reagent	+ ±	+ ±	-

The fact that substances resembling the group-specific substances A and B occur in the secretions or blood of certain animal species, and that agglutinins for A and B may be present in animal sera should not constitute a serious source of error provided that precipitin tests are made to exclude the presence of contaminating animal materials which might be expected according to the circumstances of the case. Here the use of anti-A and anti-B testing fluids prepared from immune rabbit sera has the advantage that these reagents do not react with animal blood.

From the foregoing it is evident that it is by no means always possible to make a definite diagnosis of the group of a blood stain. However, any positive findings are of value, since even though they may not permit an exact diagnosis, they limit the possibilities by excluding certain groups. If the reactions are not distinct, in view of the great responsibility involved, it will be better to withhold judgment than to render a report which may be inaccurate.

Individual Diagnosis of Stains by Means of Agglutinogens Other Than A and B. According to preliminary experiments performed by Landsteiner and Levine,²⁴ the presence or absence of the agglutinogens M

²⁴ *Jour. Exp. Med.* 47: 757 (1928).

and N in dried blood stains several weeks old was demonstrated by determining the absorbing capacity for the corresponding specific immune agglutinin

The same technic for making the absorption tests will serve as for the tests for A and B (Lauer²⁵). It must be remembered that isoagglutinins present in the blood stains can interfere with the reactions. Therefore the test cell suspension M, N and MN are taken from group O individuals. It is also important to keep in mind that particularly in the case of factor N heterozygous bloods have a lesser absorbing power than homozygous bloods (cf. page 227). The tests are controlled by performing parallel absorptions with blood stains of known types M, N and MN.

For carrying out the tests Clausen²⁶ has recommended that the absorbed testing fluid be mixed with cell suspensions of known types OM, ON and OMN in small test tubes. The mixtures are centrifuged and the supernatant fluids pipetted off and replaced by equal volumes of normal saline. The tubes are then shaken and the reactions read.

In the author's experience it has been possible to differentiate sharply blood stains containing M from those lacking this property. While it was also possible to recognize the presence or absence of N in dried blood stains, the reactions were not as clean cut so that it would seem best at least for the time being in actual medicolegal practice to restrict the examination of dried blood only to the property M. Therkelsen has obtained similar results, but remarks that not all anti M fluids tested by him gave good results for typing blood stains. Hence before making a test one should ascertain which of the anti M testing fluids available give consistent and reliable findings with stains of known types.

With regard to the differentiation of the subgroups of A, as is shown in table 102, blood stains A₁ and A₂ are distinguished by a difference in their ability to absorb anti A agglutinins. This difference is not sufficiently pronounced, however, to permit one to place full reliance on it in criminal proceedings.

STAINS OTHER THAN BLOOD

The observation that group substances are present in practically every tissue of the body and also occur in soluble form in various body secretions, has been applied in medicolegal cases when stains other than blood were found.²⁷ When examining such stains one must take into account the fact that not all individuals secrete agglutinogens in the saliva, urine, etc. E.g. if saliva containing group specific substance B is found, this would exclude individuals who do not secrete group specific substances in their saliva even though they belong to

²⁵ *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 22: 86 (1933).

²⁶ *Ztsch. f. Rassenphysiol.* 6: 49 (1933).

²⁷ Cf. Christensen *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 20: 89 (1932).

group B. On the other hand, if no group specific substances are found in a stain of saliva there are three possibilities, namely, that the saliva was derived from a group O individual, or from a "non secretor" or else that specific substances originally present have deteriorated.

Since the group specific substances, when present occur in relatively high concentration and in soluble form in the saliva or semen the group can be determined even on small quantities of such secretions. According to Schiff²⁸ the saliva present on the gummed edges of envelopes may be sufficient for a group determination. And Lattes²⁹ reports a medicolegal case in which it was possible to determine the group from traces of saliva on cigarette stubs. Other stains, such as urine and sweat stains, have a low content of group substances, and on the other hand are apt to give non specific reactions because of their high salt content. For such stains a special technic is required.

The tests on stains of secretions can be carried out either directly on the stain itself by the technic used for blood stains, or on aqueous extracts of the stains by the inhibition technic (cf page 279). The latter method is to be preferred wherever it can be applied since the tests are more readily carried out quantitatively (cf page 283), and the results are more reliable. The method of preparation of extracts of stains of secretions is based on the following properties of the group substances in secretions: (1) relative heat resistance (2) solubility in aqueous solutions and (3) non-dialyzability through cellophane or collodion membranes.

For stains containing large amounts of group substances such as nasal mucus, semen, etc., the following simple procedure gives satisfactory results. The portion of the material that has the stain is cut out or where no definite borders can be made out, the entire article e.g., a handkerchief, may be used for preparing the extract. This is placed in a beaker and extracted with an excess of boiling distilled water for ten minutes. The extract is clarified by centrifugation and then evaporated to dryness over a steam bath. The residue is dissolved in a few drops of distilled water, and this final extract, after the insoluble material has been removed by centrifugation, is tested by the inhibition technic as described on page 284.

Suitable extracts of stains of sweat and urine are prepared as follows³⁰. The stain is extracted with boiling distilled water, and this

²⁸ *Technik der Blutgruppenuntersuchung* (1932)

²⁹ *Atti IV Congresso Internaz. di Microbiol.* p. 138 (1932). Also see Cuboni *Boll. Ist. Sierot.* 7: 1 (1928); Asoda *Han-angaku Zasshi* 3: 112 (1930); Busatto *Arch. di Anthropol. Crim. e Med. Leg.* 52: 265 (1932); Moharram *Jour. Egyptian Med. Assoc.* 20: No. 11 (1937) etc.

³⁰ Cf. Yosida, *Ztschr. f. d. ges. exp. Med.* 63: 331 (1928); Schiff *Ueber die gruppenspezifischen Substanzen des menschlichen Körpers* G. Fischer, Jena (1931).

extract is concentrated to about 1/10 or 1/20 its volume on a steam bath. The concentrated extract is clarified by centrifugation and then dialyzed against running tap water for several days in a cellophane or collodion bag. The extract is then evaporated to dryness on a steam bath and the residue redissolved in a few drops of saline solution, insoluble material being removed by centrifugation. This method can also be applied to urine samples directly, e.g., when it is desired to determine the group of a suspect in a homicide case and when it is not possible to obtain a blood sample.

When stains of feces are encountered in medicolegal cases, grouping of such stains may sometimes prove of value. Hodyo²¹ and Moharram²² have described techniques for grouping feces by tests on aqueous and alcoholic extracts. These methods may prove applicable to stains of feces. Readers interested in this question should consult the original articles.

POSTMORTEM BLOOD GROUPING

As has already been mentioned, it is of value to group routinely the blood of corpses in cases of violent death for comparison with the group of blood stains, should any be found. For periods up to 24 hours after death, there is usually no special difficulty, the grouping tests are carried out as on fresh blood. When decomposition has set in, however, the problem is more difficult, and when putrefaction is advanced it may be impossible to determine the blood group any longer. The time of onset of putrefaction is influenced markedly by the temperature, so that in tropical climates, blood grouping may no longer be possible after 8 to 12 hours, while, on the other hand, if the body is stored in the refrigerator simple grouping tests may still be feasible after a week. Since postmortem bacterial invasion takes place last in the extremities, if blood taken from the heart or large vessels appears to be hemolyzed, better results may be obtained by examining samples from the peripheral vessels.

In table 104 are summarized the results of routine postmortem grouping tests carried out during the years 1939-1941 in the Office of the Chief Medical Examiner of New York City. Most of the grouping tests were made within 48 hours of death and it will be seen that only in a small percentage of cases were the tests unsuccessful.

In the present series, the frequency of group O is slightly higher and the frequency of group A slightly lower than in New York whites but this is explained by the fact that many of the bloods examined were from negroes. The distribution of the M-N types closely corresponds with that expected. Tests for the subgroups of A and AB were also carried out on 203 bloods and 46 (or 22.6 per cent) were found to belong to subgroup A₂.

It will be noticed that more A B groups are reported than M N types. There

²¹ *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 22: 95 (1933).

²² *Jour. Immunol.* 32: 229 (1937).

were two reasons for this (1) Some badly hemolyzed bloods could nevertheless be classified as group O because the serum was shown to contain both isoagglutinins α and β (2) The M N tests sometimes showed the presence of more than one type of blood because the victim had received blood transfusions before death

When grouping postmortem blood false reactions are not infrequently obtained, due to bacteriogenic agglutination. Such blood may still be grouped by testing the capacity of erythrocytes to absorb isoagglutinins (cf page 46). In some cases, as with burned bodies, pericardial fluid may be the material most easily obtainable for examination for isoagglutinins. Where suitable blood samples cannot be obtained the group may be determined from inhibition tests on aqueous extracts

TABLE 104
RESULTS OF POSTMORTEM BLOOD GROUPING TESTS

Number of Samples		Blood Group				Group Not Determined	Blood Type			Type Not Determined
		O	A	B	AB		M	N	MN	
808	Number	330	261	124	21	46	206	147	350	5
	Per Cent	45.9	34.3	16.3	3.5		28.1	20.1	31.8	

of organs, provided that the individual is a secretor. As Schiff⁷³ points out, large samples of tissues like skin and muscle or even an entire organ like the kidney or heart can be extracted with boiling water and this extract can be concentrated by evaporating it to dryness and redissolving the residue in a small volume of saline solution (cf page 321). Embalmed bodies can also be blood grouped by this technique at times especially where the preserving fluid is volatile like formalin so that it is driven off when the extracts are prepared.

Since the M N factors are restricted to the cells they usually are not determinable after hemolysis occurs.

REMARKS ON THE BLOOD GROUPING OF STAINS

Although in a certain percentage of cases individuality tests on blood stains and other stains yield positive results of obvious value these tests have been used only to a limited extent in this country mainly on account of legal difficulties.

While experienced workers will be able to distinguish between those cases which do or do not permit a diagnosis the novice may be tempted to give a definite opinion in every instance. The resulting erroneous diagnoses have led some writers⁷⁴ to the conclusion that the determina-

⁷³ *Ueber die gruppenspezifischen Substanzen des Menschlichen Körpers* Jena (1931)

⁷⁴ Gettler and Kramer *Jour Immunol* 31: 321 (1936)

tion of the group of dried stains is entirely unreliable. However, that accurate results can be obtained has been proved by the investigations of Lattes,³³ Schiff,³⁴ Therkelsen,³ Holzer,³⁵ Boyd and Boyd,³⁶ Ratcliffe,⁴⁰ Lande,⁴¹ the present writer and many other investigators.

An example of an actual case, recently tried in this country in which a gross and easily avoidable error was committed is the following:

A woman had been murdered by stabbing and suspicion centered on her stepson. The accused man was a morphine and codeine addict having acquired the habit when trying to relieve the pain in the stump of a leg lost in a railroad accident. The prosecutor's theory was that the defendant had killed his stepmother while in a fury when she withheld his supply of drugs. A few spots of dried blood were found on the sleeve of the defendant's pajamas and these were said to have come from the deceased. However, no blood grouping tests were done until some time after the crime had been committed. The defendant was found to belong to group A. The deceased had already been buried and to determine her group the dried blood stains on her nightgown and pillow were tested. The expert called by the prosecution could not demonstrate any agglutinins in these stains and without performing any tests for agglutinogens (cf. page 409) he made the unwarranted diagnosis group AB. Moreover as he could not demonstrate any agglutinins in the small spots on the prisoner's pajamas he concluded with the same fallacious reasoning that this blood also belonged to group AB and therefore must have come from the murdered woman.

At the trial the present writer explained the errors in this testimony to the jury. On inspecting the stains on the defendant's pajamas 6 or 7 small pale brown spots each about 2 mm. in diameter were found on the inside of his sleeve and only a few of these spots showed through on the outside. Therefore it was clear that the stains had resulted from the self-administration of narcotics and other shunts belonging to the defendant were found to have similar stains. The stains were not large enough to permit a grouping test.

That results can be obtained in only a certain percentage of cases is demonstrated by Lewinsky's⁴² experience with 122 examinations. In 17 cases the stains were found to be of other than human origin by means of the precipitin test. Of the remaining 105 cases the results could be used in only 12 cases because in the others (1) the defendant and murdered man belonged to the same group or (2) the unstained portions

³³ *Individuality of the Blood* London (1932)

³⁴ *Loc cit*

³⁵ *Loc cit*

³⁶ *Deutsche Ztschr f d ges gerichtl Med* 16: 445 (1931) *Ergeb d ges Med* 20: 367 (1935)

⁴⁰ *Loc cit*

⁴¹ *Jour Lab and Clin Med* 22: 191 (1936)

⁴² *Arch Path* 25: 463 (1938)

⁴³ *Deutsche Ztschr f d ges gerichtl Med* 27: 104 (1936) also see Moureau *Ann de Med lég* 16: 344 (1936). The present author has had similar experiences [*Statistical Report of the Chief Medical Examiner of the City of New York* p. 35 (1939), p. 35 (1940)]

of the clothing gave the same specific reactions as the stain itself or (3) the stain gave no specific reactions at all on account of deterioration

With regard to the legal aspect of the tests, there is nothing to prevent their prompt performance in violent death, so that the group of the murdered individual is readily determined. Nor is there any legal obstacle to the examination of the blood stains. The tests have greater value, however, when some of the defendant's blood can be obtained for the purpose of comparison. In most jurisdictions in this country, the courts do not have the power to compel defendants in criminal cases to submit to blood tests. This obstacle can only be overcome by appropriate legislation. The suggestion has been made that when a blood sample cannot be obtained from the prisoner, a urine specimen be examined for its content of group specific substances.

There is also need in this country for special medicolegal institutes such as exist in many countries abroad. By making such institutions centers for the training of experts, the utility of forensic serologic tests will be materially increased.

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APPENDIX

As was shown on page 186 the frequencies of the genes A , B and O can be calculated if the distribution of the blood groups is known by using the following formulae

$$r = \sqrt{O} \quad (1)$$

$$p = 1 - \sqrt{O+B} \quad (2)$$

$$q = 1 - \sqrt{O+A} \quad (3)$$

To facilitate the calculation of the frequencies of the genes A , B and O from those of the blood groups, nomograms have been constructed by Konorski¹ and by Boyd.² Bernstein³ has prepared a table from which the values of p , q and r can readily be obtained and Bernstein's table is reproduced here (cf. table I).

Bernstein has shown that it is also possible to calculate the frequencies of the genes from the distribution of the blood groups with the aid of rational formulae that is without the use of the square root. Bernstein's formulae are given here merely as an interesting mathematical problem rather than on account of any practical value that they may have.

$$\text{Taking} \quad A^* = A + \frac{AB}{2} \quad B^* = B + \frac{AB}{2} \quad \text{and} \quad C^* = \frac{A^* B^*}{AB} \quad (4)$$

$$\text{Then we have} \quad p, q, r = A^* B^* \left\{ C^* - \frac{A^* + B^*}{2} \right\} \quad (5)$$

The values obtained with the aid of these formulae are less accurate than those given by equations (1), (2) and (3) since the formula for C^* involves the frequency of group AB , and since the frequency of group AB is small in most populations a small chance variation in this value will cause a relatively large variation in C^* .

From equation (5) we have $\frac{p}{q} = \frac{A + \frac{1}{2}AB}{B + \frac{1}{2}AB}$. In populations where the frequency of group AB is low this would be almost identical with Hirsfeld's biochemical index given by the formula $\frac{A+AB}{B+AB}$.

As was pointed out on page 187, Bernstein's theory can be tested by calculating the values p , q and r with the aid of formulae (1), (2) and (3) and determining how closely the relation $p+q+r=1$ holds. The difference $D=1-(p+q+r)$ should not be greater than would be expected as a result of chance alone. The equation can be tested if the standard error of D is known and this is given by the formula,⁴

$$\sigma_D \sqrt{N} = \sqrt{\frac{pq}{2(1-p)(1-q)}} \quad (6)$$

where N represents the number of individuals in the sample of population examined. The value of $\sigma_D \sqrt{N}$ is readily obtained with the aid of a table constructed by Bernstein (cf. table II). To convert σ_D into the probable error of D it is only necessary to multiply by the factor 0.6745.

¹ Cited after Hirsfeld, L. *Konstitutionserologie* etc. p. 73 (1928).

² *Human Biology* 6: 558 (1934), *Ann. Eug.* 8: 337 (1938).

³ *Ztschr. f. indukt. Abstamm. u. Vererbungs.* 54: 400 (1930).

⁴ Nomograms for calculating the standard error of the expressions $1-(p+q+r)$ and $1-(m+n)$, have been constructed by Boyd [*Ann. Eug.* 8: 337 (1938)].

TABLE I

CALCULATION OF THE VALUES p , q AND r FROM THE DISTRIBUTION OF THE BLOOD GROUPS

x	\sqrt{x}	Δ	$1 - \sqrt{1-x}$	Δ	x	\sqrt{x}	Δ	$1 - \sqrt{1-x}$	Δ
01	100	41	005	5	26	510	10	140	6
02	141	32	010	5	27	520	9	146	5
03	173	27	015	5	28	529	10	151	6
04	200	24	020	5	29	539	9	157	6
05	224	21	025	5	30	548	9	163	6
06	245	20	030	6	31	557	9	169	6
07	265	18	036	5	32	566	8	175	6
08	283	17	041	5	33	574	9	181	7
09	300	16	046	5	34	583	9	188	6
10	316	16	051	6	35	592	8	194	6
11	332	14	057	5	36	600	8	200	6
12	346	15	062	5	37	608	8	206	7
13	361	13	067	6	38	616	8	213	6
14	374	13	073	6	39	624	8	219	6
15	387	13	078	5	40	632	8	225	7
16	400	12	083	6	41	640	8	232	6
17	412	12	089	5	42	648	8	238	7
18	424	12	094	6	43	656	7	245	7
19	436	11	100	6	44	663	8	252	6
20	447	11	106	5	45	671	7	258	7
21	458	11	111	6	46	678	8	265	7
22	469	11	117	6	47	686	7	272	7
23	480	10	123	5	48	693	7	279	7
24	490	10	128	6	49	700	7	286	7
25	500	10	134	6	50	707	7	293	7

Illustration Suppose that the frequencies of the blood groups are given as follows

$$\bar{O}=38\% \quad \bar{A}=41\% \quad \bar{B}=15\% \quad \bar{AB}=6\%$$

Then in the second column (\sqrt{x}) the value corresponding to $\bar{O}=0.38$ is looked for namely, $r=0.616$. In the first column the value $\bar{A}+\bar{AB}=0.47$ is located and the number corresponding to it in the fourth column ($1-\sqrt{1-x}$) gives the value of $p=0.272$. In the same way one finds that $q=0.111$ by looking for $\bar{B}+\bar{AB}=0.21$ in the first column. The table can also be used when testing the relation, $1=m+n=\sqrt{M}+\sqrt{N}$ (cf. page 239).

If the frequencies of the blood groups are given in decimals, an interpolation is carried out (Linear interpolation is sufficiently accurate). For example, if one is given the values

$$\bar{O}=38.1\% \quad \bar{A}=40.8\% \quad \bar{B}=15.2\% \quad \text{and} \quad \bar{AB}=5.9\%$$

starting with the values $\bar{O}=0.38$ and $r=0.616$, and calculating the interpolation from the relation $1/10=k/0.008$, one obtains a correction $k=0.001$, so that $r=0.617$. For $\bar{A}+\bar{AB}=x=0.467$, the corresponding value of $p=0.265+7(0.007)/10=0.270$ and for $\bar{B}+\bar{AB}=x=0.211$, $q=0.111+1(0.006)/10=0.112$.

TABLE II
CALCULATION OF THE STANDARD ERROR OF THE BLOOD GROUP RELATION, $p+q+r=1$

q	$p=0.2$	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4
0.2	0.14	0.21	0.26	0.30	0.34	0.37	0.41	0.44	0.47	0.50	0.54	0.57	0.60	0.63	0.66	0.69	0.73
0.4	0.21	0.29	0.36	0.43	0.48	0.53	0.58	0.63	0.68	0.72	0.76	0.81	0.86	0.90	0.95	0.99	1.04
0.6	0.26	0.36	0.45	0.53	0.60	0.66	0.72	0.78	0.83	0.89	0.95	1.00	1.06	1.12	1.17	1.22	1.28
0.8	0.30	0.43	0.53	0.62	0.70	0.77	0.84	0.91	0.98	1.04	1.11	1.17	1.24	1.30	1.36	1.43	1.50
1.0	0.34	0.48	0.60	0.70	0.78	0.87	0.95	1.02	1.10	1.18	1.25	1.32	1.40	1.47	1.54	1.62	1.69
1.2	0.37	0.53	0.66	0.77	0.87	0.96	1.05	1.14	1.22	1.31	1.39	1.47	1.55	1.63	1.71	1.79	1.87
1.4	0.41	0.58	0.72	0.84	0.95	1.05	1.15	1.24	1.34	1.43	1.51	1.60	1.69	1.78	1.87	1.96	2.05
1.6	0.44	0.63	0.78	0.91	1.02	1.14	1.24	1.35	1.45	1.54	1.64	1.73	1.83	1.92	2.02	2.11	2.21
1.8	0.47	0.68	0.83	0.98	1.10	1.22	1.34	1.45	1.56	1.66	1.76	1.86	1.96	2.06	2.17	2.27	2.37
2.0	0.50	0.72	0.89	1.04	1.18	1.31	1.43	1.54	1.66	1.77	1.88	1.99	2.10	2.21	2.31	2.42	2.54
2.2	0.54	0.76	0.95	1.11	1.25	1.39	1.51	1.64	1.76	1.88	1.99	2.11	2.23	2.34	2.46	2.57	2.69
2.4	0.57	0.81	1.00	1.17	1.32	1.47	1.60	1.73	1.86	1.99	2.11	2.23	2.36	2.48	2.60	2.73	2.86
2.6	0.60	0.86	1.06	1.24	1.40	1.55	1.69	1.83	1.96	2.10	2.23	2.36	2.48	2.62	2.75	2.88	3.01
2.8	0.63	0.90	1.12	1.30	1.47	1.63	1.78	1.92	2.06	2.21	2.34	2.48	2.61	2.75	2.88	3.03	3.17
3.0	0.66	0.95	1.17	1.36	1.54	1.71	1.87	2.02	2.17	2.31	2.46	2.60	2.75	2.88	3.00	3.17	3.32
3.2	0.69	0.99	1.22	1.43	1.62	1.79	1.96	2.11	2.27	2.42	2.57	2.73	2.88	3.03	3.17	3.32	3.48
3.4	0.73	1.04	1.28	1.50	1.67	1.87	2.05	2.21	2.37	2.54	2.69	2.86	3.01	3.17	3.32	3.46	3.64

If one is given the values $p=18.5$ and $r=10.8$ for example then $n\sqrt{N}=0.117$. Interpolations for p and q are made separately, and the values obtained are added together and to the value taken from the table. For example in the table, corresponding to $p=18$ and $q=10$ is the value 0.110. The interpolation for p is $5/20 \cdot 8=2$ and for q , $8/20 \cdot 12=4.8$ or approximately 5. Therefore, one has to add 0.007 to the value obtained from the table.

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